Proceedings
of the
Society
for
Experimental Biology and Medicine

Vol. 43. February, 1940. No. 2.

SECTION MEETINGS

CLEVELAND	
Western Reserve University	February 9, 1940
ILLINOIS	
Northwestern University	January 23, 1940
MISSOURI	
St. Louis University	February 14, 1940
NEW YORK	
New York University	February 28, 1940

11152 P

Preparation of Pyrogen-Free Urogastrone.*

J. S. Gray, C. U. Culmer, E. Wieczorowski and J. L. Adkison.
(Introducd by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

We have previously reported[1] that a substance which inhibits gastric secretion can be extracted from urine. The nature of this inhibitory factor is unknown, but the possibility was considered that it might represent excreted enterogastrone. Early in the course of experiments designed to test this possibility, it was discovered that the urinary extracts exerted a definite pyrogenic action. Since Meyer,

---

* Aided in part by a grant from the Committee on Endocrinology of the National Research Council.

[1] Culmer, C. U., Atkinson, A. J., and Ivy, A. C., *Endocrinology*, 1939, **24**, 631; Gray, J. S., Wieczorowski, E., and Ivy, A. C., *Science*, 1939, **89**, 489; *Am. J. Physiol.*, 1939, **126**, 507.

Cohen, and Carlson[2] have shown that artificial fever of a severe degree may practically abolish gastric secretion, it became essential to remove the pyrogen from the urinary extracts. It is the purpose of this report to show that extracts of normal urine may contain pyrogen and that the pyrogen is not identical with the gastric inhibitory factor.

Methods. Extracts of the gastric inhibitory factor were assayed in 6 Heidenhain pouch dogs injected subcutaneously with histamine. The potencies of the extracts are expressed as the percentage inhibition of free acid secretion. Rectal temperatures were taken at ½ hourly intervals for a period of 3 or 4 hours after injecting the extract. Pyrogen assays were also made by recording rectal temperature curves in a group of 5 rabbits. The extracts of normal male urine were all prepared by the benzoic acid adsorption method of Katzman and Doisy.[3]

Results. Crude extracts prepared by the original method (Method A) when injected intravenously into rabbits in doses equivalent to 30 cc of urine produced an average rise in temperature of 4.0°F. By thoroughly washing the benzoic acid precipitate in order to completely remove the mother-liquor, *i. e.,* the original urine (Method B), the average rise in temperature was reduced to 2.1°F. Additional precautions consisting of preservation of the urine with tricresol and completing the extraction process in several hours instead of several days (Method C), reduced the average temperature response to 1.0°F.

Attempts were then made to remove the remaining pyrogen by purification procedures. The results are presented in Table I. A fraction, soluble in 50% and insoluble in 75 or 87% acetone, which was obtained from crude material prepared by Method A, was found to contain considerable amounts of both pyrogen and gastric inhibitory factor. A similar fraction obtained from crude material prepared by Method B, contained only traces of pyrogen, but more gastric inhibitory factor than the above. A fraction, soluble in 70% acid (approximately N/10 HCl) acetone and insoluble in 95% acetone, which was obtained from crude material prepared by Method C, contained the gastric inhibitory factor uncontaminated with pyrogen. The substitution of alcohol (95%) for acetone in the latter procedure also yielded a pyrogen-free material, but considerable inhibitory activity was lost or destroyed with this reagent.

Discussion. The results indicate quite clearly that the gastric in-

---

[2] Meyer, J., Cohen, S. J., and Carlson, A. J., *Arch. Int. Med.,* 1918, **21**, 354.

[3] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.,* 1932, **98**, 739.

TABLE I.
Removal of Pyrogen from Urine Extracts.

Method	Purification	No. of Assays	Dose		Avg temp rise, °F	Avg gastric inhibition, %
			mg	Urine equiv., cc		
A	50-75% neutral acetone fractionation	12	3	240	2.8	—56
B	50-87% neutral acetone fractionation, repeated	6	3	660	.5	—25
,,	,, ,, ,, ,, ,,	6	6	1320	.6	—39
,,	,, ,, ,, ,, ,,	6	12	2640	.8	—55
C	70-95% acid acetone fractionation	6	3	435	0	—39
,,	,, ,, ,, ,,	6	6	870	0	—63
,,	70-95% acid alcohol fractionation	6	3	540	0	—26
,,	,, ,, ,, ,,	6	6	1080	0	—33

hibitory factor is separate and distinct from the pyrogenic factor in the extracts of normal male urine. Until such time as the gastric inhibitory factor is definitely identified, we propose to call it urogastrone.

This work is reported at this time in preliminary form because of its significance for other fields of endocrinology. It is well known that many extracts of various organs are unsuitable for intravenous administration because of fever reactions. We have assayed several commercial extracts of urine and found them to contain pyrogen. Pyrogenic substances may, in fact, be expected to occur in all extracts of biological origin which have not been treated in such a way as either to remove it or to prevent its development.[4] As long as there is no relationship between the effects of pyrogen and those of the specific component of an extract, the presence of pyrogen presents merely a problem of purification. However, when the properties of an extract resemble those of pyrogen, one should be cautious in assuming the presence of a specific component until the absence of pyrogen has been demonstrated. In view of this fact, the testing of extracts of the specific metabolic principle of the pituitary and of the antidiuretic principle of urine for pyrogen would seem to be desirable.

Conclusions. 1. Extracts of normal male urine prepared by the benzoic acid adsorption method of Katzman and Doisy contain large amounts of a pyrogenic substance, as well as the gastric inhibitory factor, urogastrone. 2. Methods for the preparaation of pyrogen-free urogastrone have been developed.

11153

Concentration and Detection of a Dye in Abscesses.

H. KROLL, SIEGFRIED F. STRAUSS AND H. NECHELES.

From the Departments of Gastro-Intestinal Research of Michael Reese Hospital, and of Physiology, University of Chicago, Chicago.

In a previous communication from this laboratory by Strauss, Neuwelt, Rovner and Necheles[1] a method was described for the detection of hidden abscesses and carcinomas. This method was based on the fact that certain dyes and colloidal substances are concentrated more or less selectively in abscesses and carcinomas. Our plan was to employ compounds which emit gamma rays and to detect the focus of their greatest concentration in the body, *i. e.*, the abscess or carcinoma, by the localization of the greatest concentration of radiation leaving the body. A sensitive Geiger-Müller counter was used and described. In experiments on dogs colloidal thorium dioxide was used as source of gamma rays and we were able to detect a high percentage of abscesses in these animals. It was realized, of course, that in the human this compound cannot be employed, because of the dangers involved.[2] It was planned, therefore, to use substances with only temporary radioactivity. In the light of our present knowledge a half-life period of radioactivity of even several days cannot be considered dangerous if sufficiently small quantities of the substance are used. Before trying out such radioactive compounds on animal or man, it was necessary to do preliminary work on their synthesis, toxicity and local concentration and the results of this work are reported here. Of the elements which are capable of being radioactivated, bromine appeared to be most promising because of its favorable half-life period, and because the reactivity of the free bromine lends itself to the synthesis of a compound which will act as a suitable carrier for the radioactive halogen. Improvements in the procedure for the concentration of the radioactive element have made it readily available in sufficient quantities for synthetic purposes.[3]

---

[4] Ivy, A. C., and Gray, J. S., *Int. Abstr. Surg.*, 1939, **69**, 409.

[1] Strauss, S. F., Neuwelt, F., Rovner, L., and Necheles, H., *Surgery*, 1938, **4**, 930.

Recent investigations have shown conclusively that certain vital dyes will localize in malignant tumors and inflamed tissue. A bisazo, acid dye, T1824, which has been used most frequently and successfully,[4, 5] proved to be of special interest. This dye possesses 2 active naphthalene radicals which are capable of undergoing a chemical reaction with bromine and thereby providing a means of introducing the radioactive atom into the body in a non-toxic form which may be concentrated in the desired area. The presence of amino and hydroxyl groups in the 1,8 positions of the naphthalene nucleus activates the positions ortho and para to these groups, enabling the dye molecule to pick up one, 2, 3, or 4 atoms very readily from an aqueous bromine solution.

A quantitative investigation of the localization process was necessary in order to determine which of the Br-dye compounds offered optimum results, *i. e.*, highest concentrations in abscesses and lower concentrations in organs and tissues.

---

[2] Laborde, S., *Presse Médicale*, 1936, **44**, 1915; Taft, R. B., *Radiology*, 1937, **29**, 530.

[3] Lu, C. S., and Ludgen, S., *J. Chem. Soc.*, 1939, 1219.

[4] Duran-Reynals, F., *Am. J. Cancer*, 1939, **35**, 98.

[5] Menkin, V., *J. Exp. Med.*, 1932, **56**, 157.

Although the literature presents ample qualitative studies on the concentrations of dyes in tissues and organs, there has been relatively little work of a quantitative nature; most investigators depending on the staining properties of the dye as a basis for determinations of the relative amounts of dye adsorbed. This means of analysis offers a serious objection in that the natural colors of the tissues and organs seriously interfere with even a visual approximation of the concentration of dye adsorbed. The addition of a bromine atom to the dye molecule permits a quantitative investigation, by the use of a suitable chemical analysis, of the actual amount of dye adsorbed per unit amount of tissue.

Experiments and Results. Preparation of dye solution. One hundred milligrams of T1824 (Evan's Blue) was dissolved in 15 ml of water. A given volume of an aqueous bromine solution, which had been previously analyzed, was added dropwise slowly, with continual stirring until the theoretical amount of bromine had been taken up.

The reaction proceeded smoothly (at room temperature) with a shifting of the color of the solution from blue to purple and finally to red-brown. If an amount of bromine greater than four equivalents was added to one equivalent of dye, precipitation of the dye took place. A sufficient amount of $NaHCO_3$ was added to neutralize the hydrogen bromide formed during the reaction and to assure a proper pH for intravenous injection of the dye solution.

Injection of Dye Solution. A suspension of streptococci and infusorial earth,* or an aqueous suspension of dogs' feces was injected subcutaneously into the right thigh of a dog. The abscess was allowed to develop for 24 hours. The dye solution, freshly prepared, was injected intravenously. In no case, did the dog exhibit any toxic effects from small doses of the dye (0.1 g). However, with much larger doses, the dye compounds with 2 and 4 Br atoms were toxic, in some cases causing death of the animal.

Analysis: The animal was sacrificed 20 hours after the injection of the dye. Samples of blood, abscess tissue, corresponding healthy tissue, spleen, kidney, liver and duodenum were taken. Examination of the internal organs showed staining of the gastrointestinal tract, spleen, and liver. The intensity of the staining decreased with the amount of bromine introduced into the dye molecule. With the monobrominated dye, the staining of the region of the abscess was apparent in a few hours.

---

* Suggested by Dr. W. Bloom of the Department of Anatomy, the University of Chicago.

The wet samples were dried overnight in an oven at 110°C. The method used for the analysis was that of Brodie and Friedman.[6] One-half to one gram samples of dried tissue were fused with NaOH in a nickel crucible. Potassium nitrate crystals were carefully added until complete oxidation of the fused organic matter was assured. The solid melt was taken up in distilled water and neutralized with concentrated sulfuric acid. The bromine, present as bromide, was oxidized to bromate by the action of sodium hypochlorite in the presence of sodium dihydrogen phosphate. The bromine concentration was then determined by the iodometric titration of the bromate present.

Results. Table I tabulates the number of milligrams of bromine found per gram of dried tissue with the various dye derivatives. It is of interest to note that with the tetra and di-substituted dye, although the amount of bromine found in the abscess was double that found in the control, spleen, liver and kidney contained an amount of bromine which was roughly twice that found in the abscess. The amount of the mono-substituted dye concentrated in abscess, liver, kidney and spleen is of the same degree of magnitude. This fact is brought out much more effectively by Table II. Selecting

TABLE I.

Concentration of Brominated Dye in Tissues as Determined by Their Bromine Contents.

	Abscess	Control	Liver	Kidney	Spleen	Intestine
A. 4 atoms of bromine per molecule of T1824. Values in mg Br/g dried tissue.						
	.0525	.0277	.0881		.117	.0260
	.0555	.0393	.0846	.115	.0898	.0458
	.0552		.151	.0886	.136	.0390
	.0616	.0144	.101		.0918	.0482
	.0682					
Avg	.0566	.0271	.106	.102	.109	.0397
B. 2 atoms of bromine per mol. of T1824.						
	.0452	.0203		.0670	.110	.0450
	.0377	.0266	.0858	.0993	.123	.0436
		.0222	.0963	.0995	.103	.0281
	.0491					
Avg	.0440	.0230	.0910	.0886	.112	.0389
C. 1 atom of bromine per mol. of T1824.						
	.0645	.0269	.0645	.0760	.0701	.0347
	.0703	.0321	.0439	.0690	.0505	.0413
	.0734					
Avg	.0694	.0295	.0542	.0725	.0603	.0380

---

[6] Brodie, B. B., and Friedman, M. M., *J. Biol. Chem.*, 1938, **124**, 511.

TABLE II.

Calculated Concentration of Brominated Dyes.

Ratio of dye* concentrated in:

	2 Br / 4 Br	1 Br / 4 Br
Abscess	= 1.57	= 4.92
Control	1.69	4.34
Liver	1.72	2.04
Kidney	1.77	2.90
Spleen	2.06	2.22
Intestine	1.95	3.84

* The relative concentration of the mono-, di-, and tetra- substituted compounds in the tissue was found by dividing the amounts of Br obtained in the analysis by 1, 2, and 4 respectively.

the tetrabrominated molecule as a standard, column one of Table II shows that nearly twice as much of the di-substituted derivative is adsorbed in the abscess as compared to the tetra-substituted one. This means that of the 3 brominated dyes the one containing 4 Br atoms showed the least concentration in the abscess.

Column 2 of Table II illustrates the corresponding ratios between the mono- and tetra-substituted compounds. The ratio approximates 5 for the abscess, and 4 for control and intestine. Liver, kidney and spleen show a decided drop, the ratio indicating only twice as much concentration of the mono-brominated dye. It is apparent, therefore, that the concentration in the abscess of the monobrominated dye is 5 times that of the tetrabrominated one.

Analysis of the blood showed that in no case was the amount of bromine present in 1 cc of blood greater than that found in one gram of control tissue. In most instances, the amount present was smaller.

In the preparation of the dye an equivalent amount of hydrogen bromide was liberated. Recent work[7] has shown that bromide ion injected into a normal animal is present in the extra-cellular fluid, and does not concentrate preferentially in any given region. It can be shown that the small amount of bromide ions thus introduced when brominated dye is injected, is distributed over the entire animal body and will produce only a slight error in our analysis which is well within the range of the maximum probable analytical error.

Discussion. An investigation of the permeability of a cell membrane to a substance must involve two considerations (1) the nature of the cell membrane, and (2) the nature of the substance. Menken[5] has shown that inflammation produced by foreign substances is characterized by certain processes; besides other changes, increased permeability and a higher acidity in the region of inflammation is observed. Duran-Reynals[4] in his studies on dye localization in tumors, has found increased permeability of certain tumor tissues to foreign proteins and dyes. The presence of a high lactic acid concentration in malignant growths has been described by Warburg.[8] The occurrence of an increased permeability and acid reaction in inflamed and tumor tissue is significant and offers a probable clue as to the mechanism of dye absorption in these tissues.

---

[7] Weir, E. G., and Hastings, A. B., *J. Biol. Chem.*, 1939, **129**, 547.

The dye T1824, in an aqueous solution is a colloid which is negatively charged because of the ionized sulfonic acid groups in the molecule. The difference in polarity between the dye molecule and the inflamed tissue will cause the dye particle to be adsorbed on the surface of the cell membrane. The amount of dye adsorbed will depend upon its colloidal size, and the permeability of the capillary wall. Therefore, other factors being constant, more of the monobrominated dye will be adsorbed than of the tetrabrominated derivative.

The retention of dye by cells may take place by a second process, that of solution of the dye in the lipoid material of the cell wall. Höber[9] has shown that symmetrical disulfonated mono-azo dyes pass through a perfused kidney, whereas unsymmetrical disulfonated dyes are retained.

The presence of a bromine atom in a dye molecule will increase the hydrophobic as well as the lipophilic nature of the dye. It is to be expected that the tetrabrominated molecule will exhibit the maximum solubility in material of a lipoid nature. In normal organs, the adsorption of a dye due to an electrostatic effect may be assumed to be constant, and the amount of dye taken up will be a function of the concentration of the dye and its lipoid solubility. This will then explain the decrease in the ratio of dye retention in Table II, column 2, for liver, kidney and spleen. This property of the monobrominated dye makes it the most suited one for our purpose of the 3 brominated compounds studied. Modifications of experimental conditions indicate that the amount of dye localized in abscess, spleen, kidney, and liver may be influenced. These modifications are being investigated in order to achieve a maximum concentration of dye in tumor or abscess tissue and a minimum in other organs.

Summary. In continuation of our work on the detection of hidden abscesses, a study of the localization of brominated derivatives of the dye T1824, in abscess tissue and organs was made. The monobrominated dye was found to be best suited for future study. It was concentrated to a much lesser degree in liver, kidney and spleen and to a somewhat higher degree in the abscess than the di- and tetrabrominated compounds. The mechanism for the localization of the dye derivatives based on physico-chemical processes is discussed.

---

[8] See Burk, Dean, On the Biochemical Significance of the Pasteur Reaction and Meyerhof Cycle in Intermediate Carbohydrate Metabolism. Page 121 of Some Fundamental Aspects of the Cancer Problem, Supplement of *Science*, Vol. 85, No. 4, New York, June, 1937.

[9] Höber, R., and Briscoe, P. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 624.

11154 P

Histological Demonstration of Vitamin A in the Human Liver by Means of Fluorescence Microscopy.

HANS POPPER.* (Introduced by A. C. Ivy.)

From the Cook County Graduate School of Medicine and the Cook County Hospital, Chicago, Ill.

It has been shown[1] that in liver and adrenal of rats there is a green fluorescence which fades during irradiation. This was attributed to presence of Vitamin A. Bodies showing morphologically similar green fading fluorescence were visualized in human organs. They gave histochemical reactions identical with those observed in normal rats or in Vitamin A deficient rats realimented with Vitamin A. These reactions are: solubility in alcohol, acetone, and mineral oil, and insolubility in water, acids, alkalis, glycerin, and reducing agents. The green fluorescence was not visible after treatment with oxidizing agents or prolonged fixation, especially at temperatures above 20°C.

The characteristic fluorescence, attributed to the presence of Vitamin A, was studied in 113 human livers by means of fluorescence microscopy. There was great variation in amount and distribution of the green fluorescing bodies. This observation agrees with chemical determinations made by Moore[2] and others. Usually the Kupffer cells showed small fluorescing droplets. After staining with phosphin 3 R they revealed silver-gray fluorescence. This was also found if staining was applied after fading of the green fluorescence. This indicated the presence of a carrier substance of lipoid character. The silver-gray fluorescence was absent after acetone treatment. In infectious diseases the number of green fluorescing Kupffer cells was generally increased. A local increase was noted on the edge of various infiltrations and sometimes in the granulation tissue of cirrhosis (5 cases). The number was diminished in starvation and acute hepatitis.

---

* Research fellow of the Cook County Graduate School of Medicine.

[1] Popper, H., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 133.

[2] Moore, T., *Biochem. J.*, 1937, **31**, 155.

In epithelial cells green fading fluorescence may be present in 4 sites. (1) It is present in fat droplets, as those which occur in physiological fatty infiltration. The smaller the droplets, the brighter was the fluorescence. In severe fatty infiltration the fluorescence was reduced. In hepatitis traces were found. (2) It is present in minute droplets which did not give a distinct Sudan 3 reaction, but which were acetone-soluble and demonstrable by phosphin 3 R. When few in number these droplets were regularly distributed along the edge of the cells as pearls on a string; larger quantities were irregularly distributed in the cytoplasm. (3) Lipofuscin revelaled a green fluorescence, but after fading or alcohol treatment a weaker red brown fluorescence appeared. This was located in the center of the cells in form of angular bodies. (4) The cytoplasm showed a slight green fluorescence which faded to blue and disappeared after treatment with lipoid solvents.

The distribution of the green fluorescence in epithelial cells varies. At present definite rules are not established. It was diminished in exhausting diseases, also in definite liver cell damage as in acute hepatitis (2 cases) or decompensated cirrhosis (3 cases). In these cases the Kupffer cells also showed little fluorescence. In obstructive jaundice (3 cases), in toxic hepatic edema and within a liver abscess the epithelial cells were poor in the fluorescence, whereas the Kupffer cells were rich. A localized absence of green fluorescence in epithelial cells was frequently observed, *e. g.,* in the dark cells at the periphery of the lobules in congested livers. The liver of newborn or premature infants showed little green fading fluorescence. In the vicinity of proliferative fluorescing Kupffer cells the epithelial cells contained many fluorescing inclusions.

The histological picture suggests that Vitamin A is transmitted from the Kupffer cells to the epithelial cells and disappears from the latter only after reduced nutrition. Sometimes the transmission to the epithelial cells is impaired with consequent retention in the Kupffer cells. Thus, observation of fluorescence permits a study of the behavior of Kupffer cells.

In addition, the characteristic green fluorescence was found in the following sites in the human body: (1) It was found in the epithelial cells of the fascicular and glomerular layer of the adrenals. The variations in the amount run parallel with that of the liver. The fluorescence in adrenals is completely absent in the newborn infant. (2) It was found in the cells of the corpus luteum of the ovary, and the tubular and Leydig cells of the testes. (3) The normal kidney did not show the fluorescence, but it was found in 2 cases of nephritis. (4) It was found in small but varying amounts in the fat cells in different organs and in fat tissue. This was not in obvious relation with the amount in the liver.

11155

Effect of Variation of Blood Pressure on the Autonomic Nervous System.

E. Gellhorn, C. W. Darrow and L. Yesinick.

From the Departments of Physiology and Psychiatry, University of Illinois, and the Institute for Juvenile Research, Chicago.

In our studies of the autonomic and somatic nervous system[1] we have frequently observed in the cat an inverse relation between blood pressure and pupillary diameter. The only reference in the literature to this phenomenon of which we are aware is a brief remark by Koch[2] that endosinusal pressure and pupillary diameter are indirectly related. Since his observations were not published in full, a brief report of our experiments seems appropriate. We have found regularly that in anesthetized cats (pentothal 40 mg/kg or chloralosane 100 mg/kg) a rise of blood pressure produced by the injection of small amounts of adrenalin (5 to 20 γ/kg) is attended by a constriction of both the normal and of the acutely sympathectomized pupil. A similar effect may be brought about by raising the blood pressure by infusion of Ringer's solution (Table I). Occasionally it was observed that the increased parasympathetic tone as evidenced by the pupillary constriction in both sympathectomized and normal pupils was accompanied by a relaxation of the nictitating membrane. If on the other hand, blood pressure was lowered by amylnitrite, a dilation of the pupil occurred. This was observed to take place both after the removal of the adrenals and after the sectioning of the cervical sympathetic. From these observations we conclude that an increase

---

[1] Gellhorn, E., and Darrow, C. W., *Arch. Internat. Pharmacodyn.*, 1939, **62**, 114; Darrow, C. W., and Gellhorn, E., *Am. J. Physiol.*, 1939, **127**, 243.

[2] Koch, E., *Klin. Wochenschr.*, 1932, **11**, 225.

TABLE I.

Cat. 2.3 kg; 40 mg pentothal/kg intraperitoneally. Blood pressure recorded from the carotid artery. Bilateral adrenalectomy. 75 cc Ringer slowly infused into the femoral vein.

Blood pressure in mm Hg	Pupillary diameter in mm
110	5.0
120	4.0
130	0.8

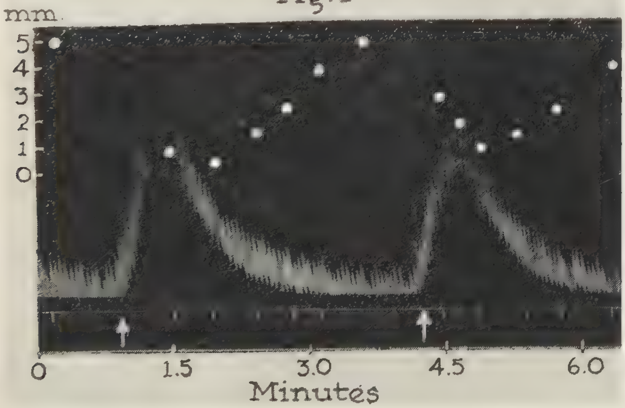
in the blood pressure leads to a decrease in sympathetic and increase in parasympathetic tone, whereas a decrease in blood pressure has the opposite effect.

Although Koch's observations show that pressure variations in the carotid sinus produce similar changes of the pupil and nictitating membrane, as we have observed after administration of adrenalin and amylnitrite, it is interesting to point out that the carotid sinus and the depressor nerves are not indispensable for this reaction. After bilateral vagotomy and denervation of the carotid sinuses, the typical effects of adrenalin and amylnitrite are retained (Figs. 1 and 2). It is not unlikely that abdominal presso-receptors are involved.[3]

The great influence which the blood pressure exerts on the pupillary diameter is further illustrated by the following examples:

Pupillary dilatation following bleeding may be greatly delayed

Fig. 1



Cat, 2.4 kg. 100 mg chloralosane subcutaneously. Bilateral vagotomy and denervation of the carotid sinuses. Upper curve: pupillary diameter in mm. Lower curve: blood pressure from the carotid artery. At the arrow, 0.0033 mg adrenalin was injected intravenously.

---

[3] Heymans, C., Bouckaert, S., Farber, S., and Hsu, F., *Am. J. Physiol.*, 1936, **117**, 619.

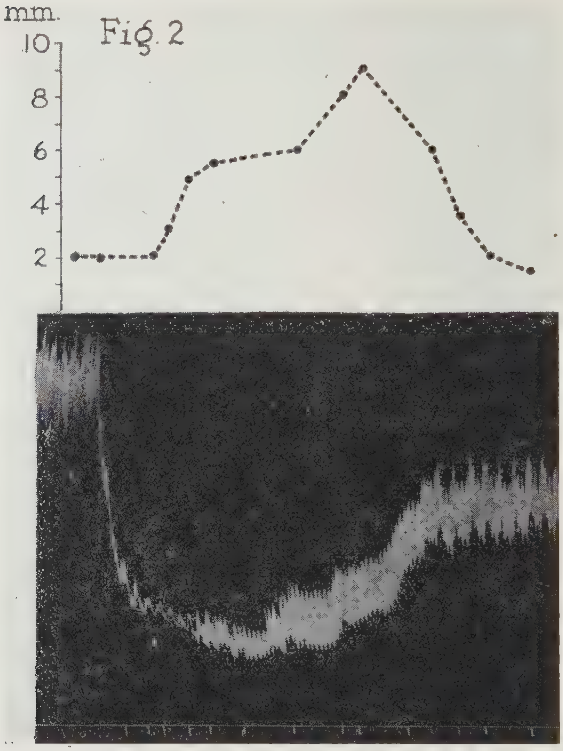
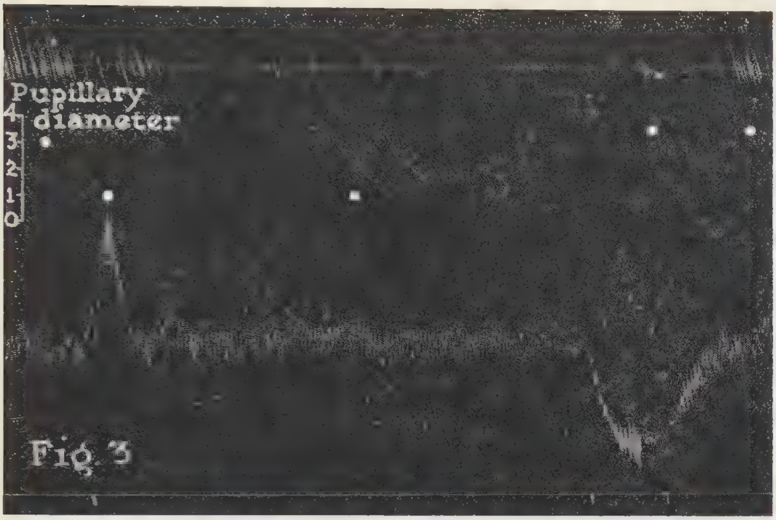


Fig. 2

Cat, 2.3 kg. Narcotized and operated as cat in Fig. 1. At the arrow, amyl nitrite is inhaled.

when the blood pressure level during the bleeding is maintained by infusion of Ringer's solution, but occurs immediately once the pressure is permitted to fall. In earlier investigations, Gellhorn and Darrow showed that the injection of metrazol is typically accompanied by a marked fall in the blood pressure and a widening of the pupils due to parasympathetic inhibition and sympathetic excitation. It was further found that in animals deprived of their buffer nerves, metrazol caused not a fall but a rise of the blood pressure. It is interesting to note that in such conditions, the pupillary dilation was markedly diminished and that if the blood pressure rise was pronounced, it led finally to the actual constriction of the pupil. This seems to indicate that the tendency of metrazol to produce pupillary dilation is inhibited by the rise in blood pressure. Nor are the effects of variations in blood pressure restricted to the autonomic nervous system for they influence the somatic nervous system as well. Koch had already noted that a rise in intrasinusal pressure led to a general



Cat, 2.3 kg. Narcotized with chloralosane 100 mg subcutaneously. Upper curve: convulsive movements recorded from the right hind leg. Middle curve: blood pressure from the carotid artery. Lower curve: first mark injection of 1 cc adrenalin 1:150,000. The second and third mark delimit the period of amylnitrite inhalation. Prior to the record, .2 cc 10% metrazol was injected intravenously.

quieting of the animal. We found likewise that adrenalin attended by a rise in blood pressure inhibits convulsions and that amylnitrite with a fall in pressure may increase them.[4] Fig. 3 illustrated this relationship. In this experiment it was observed that the pupillary diameter was 3 mm before the injection of adrenalin. It decreased to 1 mm after adrenalin and this increase in parasympathetic tone was associated with an almost complete disappearance of metrazol convulsions. In the second part of Fig. 3 the cat inhaled amylnitrite. This caused a distinct increase in frequency and intensity of the convulsions and was accompanied by a dilation of the pupil from 1 to 3.5 mm.

Summary. 1. Increase in blood pressure induced by adrenalin or infusion of Ringer's solution leads to a decrease in pupillary diameter and to a relaxation of the nictitating membrane. A fall in the blood pressure induced by amylnitrite leads to a dilation of the normal and of the sympathectomized pupil. These observations indicate that a rise in blood pressure produces parasympathetic excitation and sympathetic inhibition, whereas a fall in blood pressure has the opposite results. 2. The effects produced by variations of blood pressure are still present after bilateral vagotomy and denervation of the carotid sinuses. 3. The effects of variations of blood pressure are not restricted to the visceral nervous system but extend to the somatic nervous system, inasmuch as increase in blood pressure is associated with decreased somatic excitability and decrease in pressure is associated with increased somatic excitability.

---

[4] Gellhorn, E., Darrow, C. W., and Yesinick, L., *Arch. Neurol. and Psychiat.*, 1939, **42**, 826.

11156

Production of "Prothrombin Deficiency" and Response to Vitamins A, D and K.

MARGARET C. ELLIOTT, BERTHA ISAACS AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

This investigation was undertaken because it was found that rats receiving a diet containing 20% of mineral oil by weight developed a hemorrhagic tendency. On examining their "prothrombin time" it was found to be prolonged. It was thought that the large doses of mineral oil prevented the absorption of vitamin K as well as vitamin A and carotene.

Normal Prothrombin Time in Rats. Methods. The control diet consisted of Purina Dog Checkers and water *ad libitum.* The determination of the prothrombin time was made by a modification of Quick's[1] method. Two cc of blood obtained by cardiac puncture (22 gauge needle) under light anesthesia is drawn into 0.2 cc of 1.34% sodium oxalate solution. This is diluted to 4.0 cc with 0.9% NaCl solution and centrifuged. 0.1 cc of the diluted oxalated plasma is mixed with 0.1 cc of thromboplastin solution. 0.1 cc of 1.11% calcium chloride (anhydrous) solution is quickly added and the time for clot formation is recorded with a stop-watch. The thromboplastin solution was made by weighing, macerating and spreading fresh rat's brain on gauze so that 1 sq in. of gauze contains 0.5 g of fresh brain. This is dried and preserved at room temperature in a desiccator. When it is to be used, one of the squares is leached in 5 cc of saline solution (0.9%), and the solution incubated at 54°C for 10 min. The supernatant fluid is used. A fresh solution is made and checked for activity daily. The dried-brain-gauze preparation maintains its activity for several weeks. Chemically clean glassware was

---

[1] Quick, A. J., *J. Am. Med. Assn.*, 1938, **110**, 1658.

TABLE I.
Frequency Distribution of Prothrombin Time of Normal Rats.

Interval time in sec.	43-46	47.50	51-54	55-58	59-62	63-66	67-70
No. of cases	2	2	3	6	15	17	19
Interval time in sec.	71-74	75-78	79-82	83-86	87-90	91-102	103-106
No. of cases	18	15	14	6	2	0	1

used, and 3 determinations on each sample of blood were made and averaged. The clotting time was recorded at the first sign of formation of a coagulum. Blood samples showing hemolysis were discarded.

Results. 120 rats, weighing 200-370 g and receiving the control diet from weaning, were selected at random from our colony. The results are shown in Table I. The mean prothrombin time was 69.8 ± 0.9 (S.E.) seconds. The standard deviation of the distribution was 9.8 seconds. No significant sex difference was obtained. Of 190 cardiac punctures performed on normal rats, 12.6% proved fatal.

Effect of Repeated Cardiac Puncture. The effect of repeated hemorrhage had to be controlled in order to interpret the subsequent results. Thirty animals were placed on the control diet and their prothrombin time was determined weekly for 4 weeks. The initial average prothrombin time was 70.2 seconds, range, 43-103; 1st week, avg 69.2, range 44-93; 2nd week, avg 65.4, range 35-100; 3rd week, avg 70.1, range 59-85; 4th week, avg 71.4, range 55-86. The fourth week includes only 17 of the rats. Six of the rats were studied for 13 weeks; average 68 sec, range 61-79. The results showed that the prothrombin time of any given rat was reliable from week to week within normal limits and that no correlation exists from week to week in the rank order of the prothrombin time of individual rats. Thus weekly bleeding did not significantly affect the prothrombin time.

Effect of Mineral Oil with Agar. Method. Sixty-six normal rats, weighing 200-370 g, were placed on a diet consisting of 7 parts by weight of the powdered control diet, mixed with 3 parts of "Petrolagar". This diet contained 20% by weight of mineral oil. *Results.* The results are shown in Table II. Fifty-three of the 66 rats, or 85%, developed a prolongation of the prothrombin time within 36 days after being placed on the diet. Two of the remaining 13 did not develop a deficiency during 77 days' observation. Of the remaining 11, 4 died as a result of cardiac puncture prior to 30 days; 5 were not studied longer than 22 days, and 2 not longer than 30 days. The average control prothrombin time of the group of 66 rats was 70 seconds, range 46-90 seconds. The median prothrombin time of the

TABLE II.
Showing Effect of a 20% Mineral Oil (30% Petrolagar) in the Diet on the Prothrombin Time in Rats.

Rat No.	1	2	3	4	5	6	7	8	9	10	11
Normal P.T. in sec	80	66	66	85	85	68	80	77	60	65	66
Days on diet	22	22	22	24	24	24	21	21	28	21	21
P.T. after diet	1800+	1800+	166	204	271	485	450	103	86	1800+	1800+
Rat No.	12	13	14	15	16	17	18	19	20	21	22
Normal P.T. in sec.	66	80	74	80	73	75	79	90	70	84	71
Days on diet	23	23	20	20	16	16	16	17	17	28	28
P.T. after diet	660	1800+	1800+	600	480	1800+	780	223	286	538	201
Rat No.	23	24	25	26	27	28	29	30	31	32	33
Normal P.T. in sec	52	64	77	68	65	71	54	67	88	46	67
Days on diet	28	28	27	27	27	77	77	27	20	30	30
P.T. after diet	209	260	241	466	393	88	82	217	138	76	70
Rat No.	34	35	36	37	38	39	40	41	42	43	44
Normal P.T. in sec	67	56	82	84	73	58	67	56	61	81	68
Days on diet	22	22	30	30	30	22	22	21	21	21	21
P.T. after diet	212	67	229	209	233	192	159	145	189	133	147
Rat No.	45	46	47	48	49	50	51	52	53	54	55
Normal P.T. in sec	70	75	70	80	74	77	64	73	66	71	68
Days on diet	21	30	30	36	36	20	20	20	20	20	20
P.T. after diet	143	138	205	197	190	162	149	70	62	225	239
Rat No.	56	57	58	59	60	61	62	63	64	65	66
Normal P.T. in sec	76	62	86	60	82	65	55	48	64	68	54
Days on diet	20	20	22	22	35	21	35	20	22	22	30
P.T. after diet	149	158	245	170	129	146	154	137	93	78	100

"P.T." = Prothrombin time in seconds.
The normal average prothrombin time was 70±1.16 (S.E.) sec; range 46-90 sec.
The prothrombin time after an average of 25.4 days on the diet was 199 sec.

56 rats proven to have a deficiency between the 16th to 36th days on the diet was 199, range 118-1800+. In 7 of the 56 the prothrombin time was more than 30 minutes, which explains why the median rather than the mean of this group was used. No significant changes in weight occurred. Most of the rats remained at about their initial weight.

Effect of Mineral Oil Alone. Method. Five normal rats, weighing 200-275 g were placed on a diet consisting of 8 parts of the control diet and 2 parts of mineral oil. *Results.* The average prothrombin time on the control diet was 63.4, the range 49-74 seconds. All 5 animals developed a prothrombin deficiency within 30 days, the average prothrombin time being 365.8, the range 118-586 seconds. This experiment was done to determine whether the mineral oil in the "Petrolagar" was alone concerned.

Effect of Returning Rats with a Prothrombin Deficiency to the Control Diet. Method. A prothrombin deficiency was produced in 11 rats by the "Petrolagar diet". Then they were returned to the control diet. *Results.* The average prothrombin time of the 11 rats before they were given the "Petrolagar diet" was 77.1 seconds, range 66-90. After an average of 21 days on the diet the median prothrombin time was 480 seconds, range 103-1800+. On returning the rats to the control diet, the prothrombin time decreased to an average of 66 seconds after an average of 8.6 days.

Effect of Vitamins A, D and K Parenterally on Correcting the Prothrombin Deficiency. Method. Thirty-three rats were selected after their prothrombin time had been increased by the "Petrolagar diet". While this diet was being continued, 10 were given 50,000 I.U. of Vitamin A in oil subcutaneously by a single injection; 11 were given 500 I.U. of Viosterol in oil subcutaneously; and 8 were given 2,000 Almquist units of Vitamin K subcutaneously. A week later the prothrombin time of the rats was again determined. *Results.* The results are shown in Table III. The Vitamin A as administered had no effect. The Viosterol caused a significant improvement. The Vitamin K returned the prothrombin time to normal. Oil free of vitamin was not used because the oil containing Vitamin A caused no improvement and served as a control group for oil alone.

Discussion. It has been observed by us and others[2] that the administration of mineral oil in large amounts (20% by weight in the diet) will in time produce the manifestations of a Vitamin A deficiency. The failure of Vitamin A to return the prothrombin time

---

[2] Isaacs and Ivy, unpublished data; Dutcher, Harris, Hartzler and Guerrant, *J. Nutr.*, 1934, **8**, 269.

TABLE III.
Showing the Effect of Vitamins A, D and K on Prothrombin Deficiency in Rats.

Vitamin subcut.	No. of rats	Prothrombin time on control diet in sec		Prothrombin time on Petrolagar diet in sec		Prothrombin time 1 wk after vitamins	
		Avg	Range	Avg	Range	Avg	Range
A, 500,000 I.U.	10	70	61-81	168	132-225	202	90-340
D, 500 I.U.	11	70	48-82	182	145-235	121	70-140
K, 2,000*	8	69	52-86	279	185-590	70	55-97

* The Vitamin K is expressed in Almquist Units and was a concentrated preparation obtained from Abbott Laboratories. The Vitamin D was an activated ergosterol concentrate in oil obtained from Meade, Johnson and Co. The Vitamin A was a concentrate, 100,000 I.U. per cc, in oil obtained from Abbott Laboratories. Vitamin K-free oil was used as a diluent.

to normal indicates that the prothrombin deficiency was not due to a Vitamin A deficiency. Further work is required to show whether a deficiency of Vitamin A plays any rôle. Vitamin D was used because it has been observed to be of value for the hemorrhagic tendency in jaundiced patients.[3] How Vitamin D acts to decrease the prothrombin time is conjectural. This vitamin has been observed to cause a thrombocytosis and a shortening of the coagulation time in rats.[4] We have not made a study of the thrombocytes. The response to Vitamin K was to be anticipated. From our data it cannot be concluded whether the mineral oil produced the prothrombin deficiency by preventing the absorption of Vitamin K or by producing hepatic injury; but the latter possibility is unlikely. This method of producing a prothrombin deficiency is very convenient and has potentialities for being developed into an assay method for Vitamin K.

Summary. 1. Prothrombin deficiency has been produced in rats by feeding an adequate diet containing 20% by weight of mineral oil. 2. The subcutaneous administration of Vitamin K corrected the prothrombin deficiency; activated ergosterol definitely improved the deficiency; Vitamin A had no effect on the deficiency.

11157 P

Changes in Arterial Pressure after Bilateral Complete or Partial Ureteral Occlusion.*

R. S. MEGIBOW, L. FRIEDBERG, S. RODBARD AND L. N. KATZ.
From the Cardiovascular Department, Michael Reese Hospital, Chicago.

Recently, we have been investigating the effect of hydronephrosis on the arterial blood pressure. The blood pressure determinations in this study were made with the Hamilton manometer[1] on trained unanesthetized dogs according to a technic previously described by us.[2] In addition the blood non-protein nitrogen was determined before and after operation.

In 7 dogs complete bilateral ureteral occlusion was performed

---

[3] Gray and Ivy, *Am. J. Digest. Dis.*, 1935, **2**, 368; McNealy, Shapiro and Melnick, *Surg. Gyn. and Obst.*, 1935, **60**, 785.

[4] Phillips, Robertson, Corson and Irwin, *Ann. Int. Med.*, 1931, **4**, 1134; Tocantius, *Medicine,* 1938, **17**, 155.

* Aided by the A. D. Nast Fund for Cardiac Research.

[1] Hamilton, W. F., Brewer, J., and Brotman, I., *Am. J. Physiol.*, 1934, **107**, 427.

[2] Katz, L. N., Friedman, M., Rodbard, S., and Weinstein, W., *Am. Heart J.*, 1939, **17**, 334.

under ether anesthesia. A definite elevation in diastolic arterial blood pressure to hypertensive levels resulted in 4, and a mild increase occurred in 2 (V-24, V-26, V-28, W-13, W-19, W-20, Table I). These blood pressure elevations persisted until death in uremia 1 to 5 days following the operation. In one dog (V-87) no change in blood pressure was observed. At necropsy moderate to marked bilateral hydronephrosis was found in all the animals.

In 7 dogs partial occlusion of both ureters was carried out under nembutal anesthesia, by placing an ordinary Goldblatt clamp on a portion of the distal third of both ureters and tightening the screws to produce constriction. Four of these dogs (W-44, W-49, W-53, W-50, Table II) showed an elevation of blood pressure which was maintained for several days, the pressure then tending to return

TABLE I.
Complete Bilateral Ureteral Occlusion.

1. V-24	Syst.	140	150	165	170	170	140	(D)
	Diast.	80	90	90	100	100	80	
	Day	(C)	(1 P.O.)	(2 P.O.)	(3 P.O.)	(4 P.O.)	(5 P.O.)	
2. V-26	Syst.	150	120	(D)				
	Diast.	75	90					
	Day	(C)	(1 P.O.)					
3. V.28	Syst.	160	135	(D)				
	Diast.	85	100					
	Day	(C)	(1 P.O.)					
4. V-87	Syst.	140	175	(D)				
	Diast.	85	85					
	Day	(C)	(1 P.O.)					
5. W-19	Syst.	150	185	220	215	(D)		
	Diast.	80	105	140	135			
	Day	(C)	(1 P.O.)	(2 P.O.)	(3 P.O.)			
6. W-13	Syst.	175	150	185	(D)			
	Diast.	80	90	105				
	Day	(C)	(1 P.O.)	(2 P.O.)				
7. W-20	Syst.	115	125	120	175	(D)		
	Diast.	65	65	80	110			
	Day	(C)	(1 P.O.)	(2 P.O.)	(3 P.O.)			

Syst.—Systolic pressure mm Hg.
Dias.—Diastolic pressure mm Hg.
(C)—Control blood pressure.
(D)—Dead.
(No. P.O.)—Day post-operative.
(S.L.)—Still living.

towards the normal level. One dog still living (W-78), continues to show fluctuations between normal and moderately hypertensive blood pressure levels. Six of these animals died from intercurrent infection or were sacrificed 3 to 26 days after the establishment of the partial obstruction. In 1 of the 4 dogs (W-44), a marked increase in the blood pressure was noted after the operation, and this dog died in uremia 3 days later. At necropsy one ureter of this dog was found to be completely occluded while the other permitted the easy passage of fluid in either direction. In addition the bladder was moderately distended with urine indicating that this animal was not anuric. In the 6 other animals the blood non-protein nitrogen also rose after the operation, indicating a transient relative renal excretory insufficiency, but within a few days the non-protein nitrogen tended to return toward the normal level.

Our observations indicate that an increase in blood pressure may follow an increase in intrarenal pressure such as is produced by complete or partial ligation of both ureters. Although this rise in blood pressure is sometimes coexistent with a relative renal excretory insufficiency, it has been demonstrated previously that the two conditions are independent since the factors leading to renal excretory insufficiency are not identical with those which result in hypertension.[3]

Increased intrarenal pressure has been shown to cause a diminution in blood flow through the kidney.[4, 5] Hinman and Morison[6] noted that changes occurred in the smaller renal arteries subsequent to progressive hydronephrosis which could be the basis for a decrease in the renal blood flow, and which might therefore operate in producing chronic renal ischemia with subsequent chronic hypertension. However, we have not as yet succeeded in producing chronic arterial hypertension by this method. Our experiments suggest the possibility that hydronephrosis may be associated with hypertension on a purely mechanical basis.

Summary. 1. Complete bilateral ureteral occlusion was followed by a rise in the arterial blood pressure to hypertensive levels in 6 out of 7 dogs. Moderate to severe hydronephrosis was observed at necropsy. 2. Partial bilateral ureteral occlusion was followed by a transient rise in arterial blood pressure to hypertensive levels in 5 out of 7 dogs. Mild to moderate hydronephrosis was observed at necropsy.†

---

[3] Friedman, M., and Katz, L. N., *J. Exp. Med.*, 1938, **68**, 485.

[4] Levy, S. E., Mason, M. F., Harrison, T. R., and Blalock, A., *Surgery*, 1937, **1**, 238.

[5] Enger, R., Gerstner, H., and Sarre, H., *Zentralblatt f. Med.*, 1937, **58**, 865.

[6] Hinman, F., and Morison, D. M., *Surg. Gyn. and Obs.*, 1926, **42**, 209.

† The blood NPN determinations were made by the chemical department.

TABLE II.
Bilateral Partial Ureteral Occlusion.

1. W-47	Syst.	135	150	170	115	(D)						
	Diast.	85	85	95	65							
	Day	(C)	(1 P.O.)	(3 P.O.)	(5 P.O.)							
2. W-44	Syst.	170	175	205	190	(D)						
	Diast.	100	120	140	135							
	Day	(C)	(1 P.O.)	(2 P.O.)	(3 P.O.)							
3. W-49	Syst.	155	165	190	170	140	150	135	125	(D)		
	Diast.	85	110	105	90	105	105	65	75			
	Day	(C)	(1 P.O.)	(3 P.O.)	(5 P.O.)	(7 P.O.)	(8 P.O.)	(20 P.O.)	(26 P.O.)			
4. W-51	Syst.	150	140	(D)								
	Diast.	80	75									
	Day	(C)	(1 P.O.)									
5. W-53	Syst.	170	145	175	160	150	(D)					
	Diast.	75	85	110	95	95						
	Day	(C)	(1 P.O.)	(3 P.O.)	(8 P.O.)	(9 P.O.)						
6. W-50	Syst.	170	150	160	160	180	145	150	160	135	75	(D)
	Diast.	85	100	100	100	120	90	85	120	85	25	
	Day	(C)	(1 P.O.)	(2 P.O.)	(4 P.O.)	(5 P.O.)	(8 P.O.)	(9 P.O.)	(10 P.O.)	(12 P.O.)	(15 P.O.)	
7. W-78	Syst.	140	150	175	175	170	200	170	175	(S.L.)		
	Diast.	75	85	110	90	85	100	85	100			
	Day	(C)	(1 P.O.)	(2 P.O.)	(3 P.O.)	(6 P.O.)	(14 P.O.)	(24 P.O.)	(30 P.O.)			

Conventions as in Table I.

11158 P

Specific Action of an Antiserum for Placental Proteins on Placenta and Normal Progress of Pregnancy

HAROLD R. COHEN AND A. J. NEDZEL. (Introduced by W. H. Welker.)

From the Departments of Physiological Chemistry and Pathology, College of Medicine of the University of Illinois, Chicago.

The specific cytotoxic action of precipitins for tissue proteins has been extensively studied. Lindemann,[1] Masugi,[2] and others have demonstrated that lesions simulating glomerulonephritis were produced by injecting nephrotoxic serum into animals. Burky[3] produced lesions like those found in human endophthalmitis phacoanaphylactica by injecting rabbits with homologous lens substance. Schwentker and Comploier[4] observed that 34 of 37 scarlet-fever patients gave positive reactions to nephrotoxic serum as compared with 3 of 30 control patients, affording a possible explanation of the etiology of post-scarlatinal nephritis. Schwentker[5] produced the clinical and pathological picture of post-infections encephalomyelitis by repeatedly injecting denatured-brain emulsions into monkeys. Histological changes have been produced in the liver upon injection of antiliver sera into rabbits.

Our investigations demonstrate that the injection of an antiserum for full-term guinea-pig placentæ into pregnant guinea pigs causes them to abort.

Full-term guinea-pig placentæ were obtained directly after delivery, passed through a meat-grinder, again ground in a ball-mill, washed free of water-soluble protein and adsorbed on aluminium-hydroxide cream.[6] The placental material was then injected intramuscularly into rabbits. Blood was drawn biweekly and the sera were tested for precipitins to blood-protein. Although the finely-ground tissue had been washed free of blood-proteins to a negative precipitin-reaction, blood-protein precipitins appeared in the sera but disappeared within 2 months. This serum reacted with guinea-pig placental autolysates in a dilution of 1-1000, and with autolysates of guinea-pig liver and kidney. After removal of precipitins for liver and kidney by *in vitro* absorption, the serum still reacted strongly with the placental autolysate.

---

[1] Lindemann, M., *Ann. Inst. Pasteur*, 1900, **2**, 4; **14**, 49.

[2] Masugi, M., *Beitr. Path. Anat. u. Allg. Path.*, 1933, **91**, 82.

[3] Burky, *J. Allergy*, 1934, **5**, 466.

[4] Schwentker, F. F., and Comploier, F. C., *J. Per. Med.*, 1939, **70**, 233.

[5] Schwentker, F. F., and Rivers, T. M., *J. Exp. Med.*, 1934, **60**, 559.

[6] Marshall, J., and Welker, W. H., *J. Am. Chem. Soc.*, 1913, **35**, 820.

A total of 8 cc of this serum in 3 doses at 2-day intervals was injected intraäbdominally into each of 6 pregnant guinea pigs. Following the last injection, abortion occurred in 5 guinea pigs, in 1-5 days and every foetus was dead. Two pregnant guinea pigs injected with normal rabbit serum did not abort.

The abortive effect of the antiplacental serum is apparently due to its effect on placental tissue. Placentæ obtained after abortion showed the following histological changes in contrast with normal placentæ obtained from the control animals. There was a definite increase in blood volume (passive congestion), spasm of the uterus, flattening of the syncitial cells, eosinophilia, and signs of necrosis of the formed elements, especially in the space between the maternal and foetal parts of the placenta (hyalinization, karyorrexis, karyolysis, cytolysis, pyknosis). No changes were found in the liver or kidney.

In the case of one of the 6 pregnant guinea pigs that had been injected with antiplacental serum, no certain knowledge exists of its abortion. That the animal may have aborted is indicated by the large loss of weight and the existence of cellular debris in the uterine cavity. Histological examination of the liver and uterus revealed definite histological changes. No lesion was found in the kidneys.

The blood sera of 2 women known to be habitual aborters gave positive precipitin-reactions with proteins prepared from full-term human placentæ.

Conclusions. An antiserum for full-term guinea-pig placentæ reacted with liver and kidney autolysates. These precipitins were removed by *in vitro* absorption. The serum still reacted strongly with placental autolysates. Injection of this serum into pregnant guinea pigs caused them to abort.

11159

Studies on Preserved Human Blood. V. Decrease in Prothrombin Titer During Storage.*

E. D. WARNER, ELMER L. DEGOWIN AND WALTER H. SEEGERS.

From the Department of Pathology and the Department of Internal Medicine, State University of Iowa, Iowa City.

Recent work has shown that a deficiency in plasma prothrombin is the cause of a bleeding tendency in a number of conditions, most notable of which are disease of the biliary tract, hemorrhagic disease of the new-born, and certain types of liver injury. For patients with such disturbances, blood transfusion is often necessary as an emergency measure. When fresh blood is given to the patient, there can be little doubt that the prothrombin in the transfused plasma can be utilized, and, although greatly diluted by the plasma of the recipient, may be of at least temporary benefit. With the rapidly increasing use of preserved blood for transfusions, the stability of prothrombin under conditions of storage has become a problem of considerable importance.

Rhoads and Panzer[1] reported that the prothrombin content of stored blood, as determined by the method of Quick,[2] diminishes very rapidly, so that after a few days of storage such blood would be useless for treating prothrombin deficiency. In contrast Belk, Henry and Rosenstein[3] found only moderate prolongation of Quick's prothrombin time at 10 days and no prolongation after 5 days of storage. More recently, Lord and Pastore[4] have studied the disintegration rate of the plasma prothrombin in preserved blood by the quantitative prothrombin method of Warner, Brinkhous and Smith.[5, 6] Their results are much more favorable and correspond very closely with the results which we have obtained. In the present paper data are

---

* Aided by a grant from the John and Mary R. Markle Foundation. Funds for technical assistance were also supplied by the Graduate College, State University of Iowa.

[1] Rhoads, Jonathan E., and Panzer, Lillian M., *J. A. M. A.*, 1939, **112**, 309.

[2] Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

[3] Belk, William P., Henry, Norman W., and Rosenstein, Florence, *Am. J. Med. Sci.*, 1939, **198**, 631.

[4] Lord, Jere W. Jr., and Pastore, John B., *J. A. M. A.*, 1939, **113**, 2231.

[5] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

[6] Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

presented on the rate of prothrombin disintegration in blood stored at 3° to 5° in a dextrose-citrate mixture and in sodium citrate alone. These data show that the prothrombin content of the plasma decreases very gradually, reaching 50% of normal in about 3 weeks of storage. The disintegration rate was essentially the same in both preservatives studied.

Procedure. Blood was drawn from 6 healthy adult males into each of 2 solutions. One mixture consisted of 23 volumes of blood and 2 volumes of 3.2% dihydric sodium citrate in distilled water. The other mixture, previously described,[7] was composed of 10 volumes of blood, 13 volumes of 5.4% anhydrous dextrose in water, and 2 volumes of 3.2% dihydric sodium citrate in water. The two blood mixtures, from each of the 6 donors, were apportioned aseptically into a series of cotton-plugged test tubes and stored at 5°. At 5-day intervals a test tube from each series was taken from storage, the blood-mixture centrifugalized at 5° and the plasma titrated for prothrombin by the method of Warner, Brinkhous and Smith.[5, 6]

Preliminary observations indicated need for a slight modification of the usual method of titration. The test was carried out by comparison of unknown plasma with fresh human plasma of known potency. It was found, in the present study on preserved blood, that plasmas containing high concentrations of dextrose, as a diluent, tended to give distorted values when compared with citrated plasmas. The practice was therefore adopted of comparing the preserved plasmas with controls of fresh plasma diluted in the same manner and with the same substances as were the stored bloods. In all cases we adhered to the usual practice of adding a small amount of acacia during titration, to stabilize the colloidal state of the mixture.[6]

Results. A comparison of the values obtained in the 2 types of preservatives revealed no difference in the rate of disintegration, although hemolysis proceeded much more slowly in the dextrose-citrate mixture than when citrate alone was used. In Fig. 1 progressive prothrombin values obtained on 12 samples of stored blood are plotted as individual curves. The curve of the mean goes steadily downward, the prothombin reaching the 50% level between 21 and 25 days of storage. After 10 days of storage the mean prothrombin value was still 91% of normal, as compared with 20% obtained by Rhoads and Panzer, who used the method of Quick.

When Lord and Pastore[4] used Quick's method rather than the 2-stage method they likewise obtained low readings. However, Belk,

---

[7] DeGowin, E. L., Harris, J. E., and Plass, E. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 126.

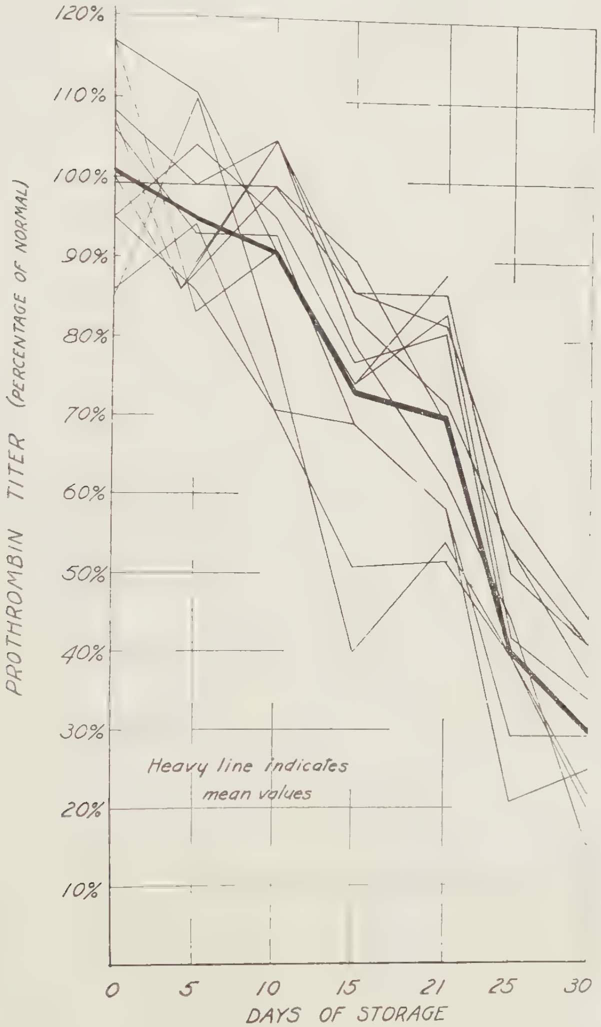


Fig. 1.
Prothrombin Content of Stored Human Blood.

Henry and Rosenstein[3] found only moderate prolongation of Quick's prothrombin time after 10 days of storage. We have used Quick's prothrombin time test on a number of samples of stored blood ranging from 0 to 30 days of age and the results, although rather variable, were only slightly lower than those obtained by the 2-stage method. No instances were found in which the value fell to less than 40% of the control level in 3 weeks of storage. It is possible that slight differences in conditions of storage, or in the anticoagulant used, may account for the differences in results which have been obtained with Quick's prothrombin test.

Discussion. The quantitative estimation of the prothrombin content of blood is necessarily an assay technic based upon the physiological activity of the thrombin formed from the prothrombin, and no standard method has been generally adopted. Some of the reasons for a disparity in the results obtained by the prothrombin time method of Quick and by the titration procedure of Warner, Brinkhous and Smith have been discussed elsewhere.[8] The latter method eliminates errors due to factors which alter the time required for thrombin formation. Thus, this method at least approximates the ideal of eliminating all variables other than the amount of prothrombin present. In contrast, the prothrombin time of Quick represents a summation of factors which determine the speed with which the blood can clot. It should be pointed out, however, that such a measure of the coagulability of the blood may be of great practical use. It is possible that slight alterations in the reactivity of prothrombin in stored blood may occur under certain conditions and that this may detract greatly from the utility of the transfused prothrombin, even though the total amount present has not appreciably decreased. Clinical evidence will help in answering this question.

Summary. The rate of disintegration of the plasma prothrombin in preserved blood stored aseptically at 5° was studied by the quantitative prothrombin method of Warner, Brinkhous and Smith.

The prothrombin values were found to decrease very gradually, reaching the 50% level at the end of about 3 weeks.

No difference in the rate of disintegration was found between blood stored in sodium citrate and that stored in a citrate-dextrose mixture.

---

[8] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296; PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 197.

11160 P

Vital Staining of Virus Lesions on Chorio-Allantoic Membranes by Trypan Blue.

Jean V. Cooke and Russell J. Blattner.

From the Department of Pediatrics, Washington University School of Medicine.

In the study of the early focal lesions of viruses on chick chorio-allantoic membranes, some difficulty is often experienced in recognizing the earlier focal lesions on direct examination of the membrane even with moderate magnification. The recognition of the foci and the preservation of such membranes for more leisurely study under the microscope has been greatly facilitated by staining the lesions with trypan blue, and, after formalin fixation, mounting them in glycerin gelatin.

In this method, 1 cc of a 0.5% aqueous suspension of trypan blue is employed. It is placed directly upon the membrane when ready for examination through the window in the shell. Gentle rotation of the egg facilitates the general distribution of the stain, after which the shell is closed and the egg placed in the incubator for from 10 to 30 minutes depending on the age and extent of the lesions. The more extensive lesions require a shorter time to take up the stain and may become too intensely colored for satisfactory examination if the stain is left on too long.

The membrane is then removed in the usual manner, washed gently in physiological saline to remove the excess of trypan blue, and fixed flat in 10% formalin for a few minutes. After draining away the excess liquid the membrane is flattened on a 2 x 2.5 inch glass slide and mounted in a glycerin gelatin containing 50% glycerin, 5% gelatin, and 1% phenol. The gelatin should be soaked in water about 2 hours before adding the glycerin and phenol, then heated gently for a few minutes while stirring. For mounting the membrane, the glycerine jelly is melted and while still hot (about 70°C) dropped on the membrane until it is well covered, flaming the slide gently but not enough to produce bubbles. It is then mounted with a warmed cover slip and gentle pressure applied for a minute or two until the jelly has begun to set. When fully hardened the excess jelly is removed and the edges sealed with Canada balsam or asphalt cement.

Under the low power of the microscope uninoculated membranes or those to which simple broth or sterile serum has been added 24 to 48 hours previously, show that very little trypan blue is taken up by the cells, although sometimes certain of the ectodermal cells and sometimes sheets of them have absorbed some of the stain. Cells around traumatized areas also are often stained. The foci of proliferation in virus lesions, however, stand out prominently as more or less isolated clumps of well stained cells which are easily recognized from their distinct focal arrangement.

Most of our observations have been made with the lesions of the St. Louis type of encephalitis virus in which it is usually possible to recognize the virus foci in the vitally stained membrane in 18 hours and always in those 24 hours old. The focal lesions from vaccinia and variola virus also are well stained by trypan blue and easily recognized. The finer cell structure of the virus lesion is not easily made out in the thickened membrane, but the cells show well in paraffin imbedded sections.

11161 P

A Consideration of Extra-Valvular Elements in the First Heart Sound.

JOHN R. SMITH, WM. B. KOUNTZ AND ARTHUR S. GILSON.

From the Departments of Internal Medicine and Physiology, Washington University School of Medicine, and the Barnes Hospital, St. Louis.

The relation between auricular systole and the first sound, the rôle of the atrio-ventricular valves, and myocardial contraction have been considered, together and separately, as factors contributing to the production of the first heart sound.[1–7] Some observers have felt that closure and tensing of the atrio-ventricular valves at the onset of systole are the sole elements in causing the first sound, while others have adhered to the theory that the initial heart sound is partly muscular and partly valvular in origin. We investigated the problem, applying a method designed to prevent atrio-ventricular valve movement.

The instrument here used to record the heart sounds was a cathode-ray stethograph, furnished by the Burdick Corporation. The instrument is aperiodic to most vibrations produced by the heart, and the

---

[1] Eckstein, R. W., *Am. J. Physiol.*, 1937, **118**, 359.

[2] Wiggers, C. J., and Dean, A. L., *Am. J. Physiol.*, 1916, **12**, 476.

[3] Wiggers, C. J., *Arch. Int. Med.*, 1919, **24**, 471.

[6] Palfrey, F. W., *New England J. Med.*, 1929, **200**, 1917.

[7] Dock, W., *Arch. Int. Med.*, 1933, **51**, 737.

lags and overshoots, so characteristic of most string galvanometers used for this work, are lacking. Therefore, the sound tracings are more nearly exact reproductions of the cardiac vibrations produced. The instrument is so constructed that sound waves below fifty cycles per second, which are not appreciated by the normal ear, may be heard.

Dogs were anesthetized with veterinary nembutal. The thoraces were opened and respiration was maintained by a respiratory pump. To prevent movements of the mitral and tricuspid valves, small balloons were affixed to the ends of brass tubes 3 mm in diameter; these were introduced into the ventricular cavities through small openings made in the auricles and then through the mitral and tricuspid apertures. The inflow of blood into the heart was then cut off by clamping the venae cavae, so as to empty the heart of blood, and the balloons were inflated by water or air to a pressure of 70 mm of Hg against a manometer. While this inhibited all movement of the auriculo-ventricular valves, the myocardium remained intact and could contract against the resilient balloons. Sounds arising from both ventricles were recorded by applying the transmitter of the stethograph directly to the myocardium at the intraventricular septum just superior to the apex. Auscultation was also done by placing an ordinary stethoscope on the myocardium. Small doses of barium chloride were administered to these hearts to forestall ventricular fibrillation.

Control readings with simultaneous electrocardiograms were obtained on each animal before the experimental procedures were begun. When the "intraventricular" balloons were in place and inflated, the character of the sounds was profoundly changed, but systolic vibrations were recorded, and were heard on direct auscultation. It must be remembered that the inflated balloons filled the ventricular cavities without visibly distending them. The first sound on auscultation had lost much of its "normal" quality, but persisted as a dull snapping sound during the time the balloons were inflated.

Constriction of the auriculo-ventricular ring was carried out by drawing as tightly as possible a ligature passed around the auriculo-ventricular sulcus as done by Dock;[7] this prohibited any flow of blood into or out of the ventricles and immobilized the atrio-ventricular valves. In a vigorously beating heart in good condition, systolic sounds could be heard during the period of auriculo-ventricular constriction, and were very similar to those obtained when the intraventricular balloons were used. They were dull, snapping sounds. The second heart sound, of course, completely disappeared. In some hearts—those that had been subjected to previous manipulation, and in which the myocardium may have been weakened or damaged—

constriction of the auriculo-ventricular ring caused a cessation of all heart sounds. Dock stated that constriction of the auriculo-ventricular ring invariably caused complete cessation of the heart sounds. Our results, therefore, were only in partial agreement with his.

During the onset of ventricular systole it would seem that, sudden change of intraventricular pressure would tense all structures subjected to the pressure. In the vigorously contracting myocardium this tensing is such as to produce audible sounds in the absence of motion of the ordinarily movable valve structures.

11162 P

Anesthetized Kittens Used to Test for Staphylococcus Enterotoxin.

N. M. Phatak and C. F. Pentler. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, and the Department of Pharmacology, University of California Medical School, San Francisco.

The reaction of kittens to staphylococcal enterotoxin was described by Dolman, Wilson and Cockcroft,[1] and confirmed by Kupchik[2] and Minett.[3] It was shown that kittens will vomit after intraperitoneal injection of heated staphylococcal filtrates that contain enterotoxin.

We find that this test can be rendered more objective and more convenient by anesthetizing the kittens with sodium pentobarbital. The kittens are given a small meal; after 30 minutes they are injected intraperitoneally with sodium pentobarbital. The dosage is 30 mg per kg body weight, as a 5% aqueous solution. This produces light anesthesia, which lasts for several hours. The heated staphylococcal filtrates are injected intraperitoneally after the onset of anesthesia; then the kittens are laid out in a row and observed for vomiting. Only definite vomiting with expulsion of vomitus, between 20 and 90 minutes after injection of the filtrate is considered as positive.

The kittens should be kept warm, and turned over every hour to avoid hypostatic congestion. It is easy to handle a large number of kittens in this manner. Sensitivity is somewhat reduced, but specificity may be increased since the effects of excitement are avoided. It is important to have shallow anesthesia, and to use 50% larger doses of filtrates than when working with conscious kittens.

---

[1] Dolman, Wilson and Cockcroft, *Canad. Pub. Health J.*, 1936, **27**, 489.

[2] Kupchik, *J. Infect. Dis.*, 1937, **61**, 320.

[3] Minett, *J. Hyg.*, 1938, **38**, 623.

11163

Inactivation of Methyl Testosterone in Castrate Male Rats.

GERSON R. BISKIND. (Introduced by Charles Weiss.)

From the Department of Pathology, Mt. Zion Hospital, San Francisco, and the Division of Pathology, University of California Medical School, San Francisco.

Methyl testosterone has been shown to be more effective on oral administration in man[1] and rats[2] than testosterone, testosterone propionate, and other related semi-synthetic androgens. This may indicate either a different route of absorption from the intestinal tract, or a different site of inactivation. To examine these possibilities, pellets of crystalline methyl testosterone* were implanted into the subcutaneous tissues, the normally situated spleen, and the transplanted spleen with pedicle tied, of groups of large castrated adult male rats of the Long-Evans strain. In the last group the spleen was transplanted at the time of castration between the overlying muscle and skin without impairing its vascular supply. Three weeks later the pellet was placed in the spleen, and at the same time a ligature was tied around the splenic vessels, which probably did not completely obstruct the artery. In all groups the pellet was inserted three weeks after castration. The animals were sacrificed 4 weeks after the pellets were implanted, the genital tract was removed, the seminal vesicles, prostate and bulbo-urethral muscles dissected free, and each of the organs was weighed on a damped balance. The contents of the seminal vesicles were carefully expressed before weighing. The remainder of each pellet was removed, cleaned, dried and weighed.

With the pellet implanted subcutaneously, the weights of the respective organs of the group show the usual significant increase when compared to the castrate control group, indicating that effective absorption from the pellet has taken place. In the group with the normally situated spleen, the weights of the respective organs are of the same order of magnitude as the castrate control group. The slight variation is explicable by the fact that the average weight of the animals in the former group is considerably more than in the latter group.

In Group IV, where the pellet was inserted in the transplanted spleen, the weights of the respective organs are of the same order of magnitude as in the group with the pellet in the subcutaneous tissues, indicating that absorption has taken place in the spleen, and that when the circulation from the spleen is shunted away from the portal into the general circulation enough is absorbed to be capable of exerting specific androgenic action. The average amount absorbed from any site from each pellet is .089 mg per day, which is greater than that absorbed from similar pellets of testosterone propionate, which averages .065 mg per day.[3]

---

[1] Foss, G. L., *Brit. Med. J.*, 1939, **2**, 11.

[2] Miescher, K., and Tschopp, E., *Schweiz. Med. Wschr.*, 1938, **68**, 1258.

* Supplied through the courtesy of Dr. R. C. Mautner, Ciba Pharmaceutical Products, Inc.

Thus the same interpretations apply to this experiment as to the similar one in which testosterone propionate was tested;[3] namely, that transportation of the methyl testosterone through the liver from the normally situated spleen causes its inactivation. This experiment, like the previous one, has not completely excluded the possibility that the normal spleen may play a minor part in this process. Slight histological changes occur in the transplanted spleen which may conceivably be associated with altered function.

The results of the present observations indicate that the quantita-

TABLE I.
The Inactivation of Methyl Testosterone in the Castrate Adult Male Rat.

Animal No.	Seminal vesicles, mg	Prostate, mg	Bulbo-urethral muscles, mg	Animal weight, g	Pellet absorption, mg
I. Castrate controls					
1840	103	125	270	240	
1845	86	135	442	340	
1846	104	65	484	344	
1847	195	133	597	360	
1850	174	200	581	360	
1851	177	249	689	360	
1857	161	144	637	360	
Avg	143	150	528	338	
II. Methyl testosterone implantation in subcutaneous tissues					
1856	524	761	1315	380	.075
1858	498	659	1094	360	.091
1859	424	750	1214	250	.082
Avg	482	723	1208	330	.083
III. Methyl testosterone implantation in normally situated spleen					
1853	185	150	511	410	.078
1854	244	124	642	460	.107
1855	133	146	455	400	.105
Avg	187	140	536	423	.097
IV. Methyl testosterone implantation in transplanted spleen					
1838	410	440	900	340	.110
1839	707	1290	1255	470	.090
1843	640	910	1210	400	.060
1844	423	665	690	376	Broken
Avg	545	826	1014	396	.087

---

[3] Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

tive differences in specific activity noted on oral administration of testosterone propionate and methyl testosterone may be related to a difference in the route of absorption from the intestinal tract, rather than to a lesser destruction of methyl testosterone by intestinal ferments as has been suggested.[2] If absorption is mainly by way of the lacteals and lymphatics, thus avoiding the liver, a greater specific effect should be present than if absorption occurs into the portal circulation. It is likely that the specific chemical structure influences the route of absorption. There is one other possibility, although a slight one. Methyl testosterone may be absorbed through the esophagus during ingestion and thus enter the systemic circulation by way of the esophageal veins; or if it is less rapidly destroyed in the gut, or less rapidly absorbed, enough might reach the lower gut to enter the systemic circulation by way of the hemorrhoidal veins.

Summary and Conclusion. Methyl testosterone, like testosterone propionate, does not exert its specific effect on the genital organs of castrate male rats when implanted in pellet form in the spleen. When the pellet is placed in the transplanted spleen, with the splenic vessels ligated, the specific effect returns. As both androgens appear to be destroyed in the liver, it is suggested that the differences in the specific effects of these two substances when administered orally may be due to different routes of absorption from the intestinal tract (*e. g., via* the lymphatics), rather than different sites of inactivation.

11164

Proof of Excitability of Mammalian Ventricles During Systole.

René Wégria. (Introduced by Carl J. Wiggers.)

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

In a recent paper[1] we reported that a single, short, strong, localized shock applied directly to different regions of the left ventricle of dogs anesthetized by sodium barbital can produce permanent ventricular fibrillation. The same results have since been obtained on dogs under chloralosane and in cats under dial anesthesia. A shock, if strong enough and given during the vulnerable period near the end of ventricular systole, produces one extra-systole followed by about 4 more or less coördinated beats and then by true ventricular fibrillation. If the shock is not quite strong enough or comes a little early in systole, instead of producing ventricular fibrillation, it gives a single premature contraction and only rarely several.

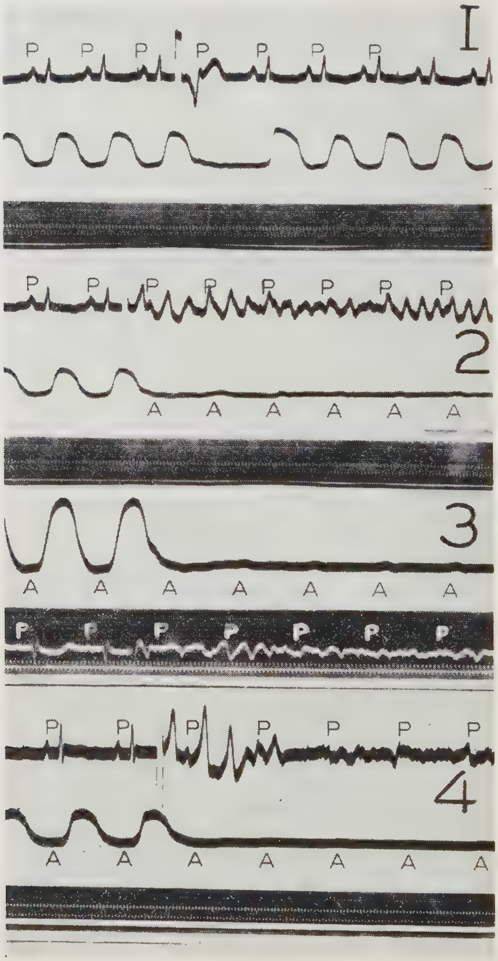
---

[1] Wiggers and Wégria, *Am. J. Physiol.*, in press.

It is important to establish with certainty that these effects are due to ventricular excitation by the shock applied during systole rather than to a spread of current to the atria and subsequent ventricular excitation by supraventricular impulses arriving during diastole.

By recording simultaneous electrocardiograms and left ventricular pressure curves the latter possibility can be definitely excluded for the following reasons:

1. The premature electrical variation starts too shortly after the stimulus to allow for the A-V conduction time (Figs. 1-4).



2. The electrical variation has the form of a ventricular premature beat and not that of a ventricular beat due to an auricular impulse (Figs. 1-4).

3. The P-wave occurs after the premature ventricular complex and the spacing of the P-wave remains normal, showing that the auricular rhythm has not been disturbed (Figs. 1, 2-4).

4. The premature contraction when not producing fibrillation is followed by a full compensatory pause which, although possible in case of auricular premature systoles, is a rare occurrence (Fig. 1).

5. A stimulus applied a little too early in ventricular systole to produce an extra contraction never induces premature auricular contraction as it should if it were spreading to the atria.

When ventricular fibrillation follows as in Figs. 2, 3, 4, the undisturbed and regular sequence of the P waves and of small pressure elevations of atrial origin shows that the stimulus acts directly on the ventricle.

The conclusion is reached that a single short strong localized shock applied during the latter portion of systole is directly responsible for the premature contraction or ventricular fibrillation.

11165 P

A New Reagent for Quantitative Estimation of Estrone.

Clara M. Szego and Leo T. Samuels.

From the Department of Physiological Chemistry, University of Minnesota, Minneapolis.

Most present colorimetric methods of assay[1-4] for the estrogenic hormones have two serious defects: a lack of specificity and the production of interfering colors with inactive impurities. Bachman,[5] however, has recently reported a modification of the Kober reaction which appears to be specific for estriol.

This report deals with an investigation of guaiacolsulfonic acid:

---

[1] *a.* Kober, S., *Biochem. Z.*, 1931, **239**, 209—modified by: *b.* Cohen, S. L., and Marrian, G. F., *Biochem. J.*, 1934, **28**, 1603; *c.* Cartland, G. F., Meyer, R. K., Miller, L. E., and Rutz, M. H., *J. B. C.*, 1935, **109**, 213; *d.* Venning, E. H., Evelyn, K. A., Harkness, E. V., and Browne, J. S. L., *J. B. C.*, 1937, **120**, 225; *e.* Bachman, C., *J. B. C.*, 1939, **131**, 455.

[2] Zimmerman, W., *Z. physiol. Chem.*, 1935, **233**, 257; 1936, **245**, 47.

[3] Smulovitz, M. J., and Wylie, H. B., *J. Lab. and Clin. Med.*, 1935, **21**, 210.

[4] Kober, S., *Biochem. J.*, 1938, **32**, 357.

[5] Bachman, C., *J. B. C.*, 1939, **131**, 463.

$(CH_3.O)$ (HO) $(C_6H_3.SO_3H)$, substituted for phenolsulfonic acid as the color reagent in the Kober[1] reaction. The reagent was prepared by dissolving the potassium salt, thiocol, in concentrated sulfuric acid.

Conditions for the reaction with crystalline estrone* in both 1.0 cc and 7.5 cc final volumes have been studied.

Guaiacolsulfonic acid gave a pink color with estrone which was similar in intensity to that given by phenolsulfonic acid. The resultant color of the reaction in the 1 cc final volume was more stable than that given by the Cartland[1] phenolsulfonic acid procedure. The color in the 7.5 cc final volume was at least as stable as that given by the Venning[1] phenolsulfonic acid procedure modified to that volume.

Beer's law applied to the reaction with the new reagent over at least 25-fold variation in hormone concentration.

A 10% solution of thiocol and concentrated sulfuric acid was found to be most satisfactory for the 1 cc procedure. This procedure involves the same series of steps as the Kober[1] reaction except that the optimum times for each step were as follows:

1. Six minutes initial heating in the boiling water bath.
2. Cooling in ice water 5 minutes.
3. After dilution, heating in a boiling water bath for 7 minutes.
4. Cooling again in ice water for 5 minutes before the final dilution.

Concentrations of guaiacolsulfonic acid were compared for the reaction in the 7.5 cc volume and it was found that 20% thiocol was more satisfactory than the 10% reagent used in the 1 cc technic. A one-minute initial heating time, and a 2-minute reheating time proved best for this amount. Except for reagents and time conditions as given, volumes of reagents for the 7.5 cc procedure were one-half those used by the Venning group,[1] and the technic of color development was the same.

The error in the determination was less than a maximum of ±5% with $5\mu g$, and ±2% with $15\mu g$ quantities of estrone. As little as 1 μg in a final volume of 7.5 cc could be detected.

The guaiacolsulfonic acid reagent is less viscous than the phenolsulfonic acid reagent, making pipetting easier. The reproducibility of the new reagent is better than that of the latter because thiocol is easily weighed and is not hygroscopic.

It reacts with estrone and at slower rate with estriol, but not with estradiol, androsterone, sodium pregnandiol glycuronidate or cholesterol. Phenolsulfonic acid on the other hand, reacts with estrone, estriol, and estradiol.

---

* Estrone and estriol for this study were kindly supplied by Parke, Davis and Company. Estradiol and androsterone were furnished by the Schering Corporation. Equilen and equilenin were given us by Prof. Oskar Wintersteiner of Columbia University.

As mentioned above, the reaction with estrone is complete in one minute while estriol requires longer initial heating to give a significant color. The rate of reaction of guaiacolsulfonic acid with estriol indicates a preliminary dehydration to estrone in the hot concentrated sulfuric acid during the initial phase of the reaction, the estrone so formed subsequently taking part in the dye complex. It was noted above that there is no significant reaction with estradiol, a compound which has the same structure as estriol except that the hydroxy group on the 16-carbon is missing. The two adjacent hydroxy groups on the 16, 17 carbons of estriol have been shown to be converted to a 17-ketonic group under dehydration conditions.[6]

The reaction of the guaiacolsulfonic acid reagent with equilin and equilenin in the 7.5 cc technic is faintly positive when a 6-minute initial heating time is used. On the other hand, as with estriol, the color is negligible at a 1-minute initial heating time.

Under the conditions standard for the 7.5 cc technic (one minute initial heating and 2 minutes reheating), therefore, the guaiacolsulfonic acid reaction appears to be specific for estrone. This greater degree of differentiation may be of importance in the possible application of the method to mixtures of the estrogenic hormones. Its usefulness may become more apparent as advances in purification methods make possible reliable studies upon urine and tissue extracts.

11166

Observations on Human Blood Stored at 4° Centigrade.

WILLIAM F. LIPP AND ROGER S. HUBBARD.
From the Buffalo General Hospital, Buffalo, N. Y.

A study was made of citrated blood preserved for transfusion purposes. Two 500 cc quantities of blood were collected from apparently healthy donors under aseptic conditions. In each instance, 50 cc of a 2.5% solution of sodium citrate were used to prevent coagulation, bringing each specimen to a total of 550 cc of the blood-citrate mixture, approximately a 9% dilution of the blood.[1] The blood was stored in sealed flasks at a temperature of 4°C.[2] On the days the tests were made, the flasks were removed from the refrigerator, agitated for 3-4 minutes, the required amount of blood withdrawn, and the flasks sealed and returned to the refrigerator.

---

[6] Butenandt, A., and Hildebrandt, F., *Z. physiol. Chem.*, 1931, **199**, 243.

[1] Lewisohn, R., *Med. Rec.*, 1915, **87**, 141.

[2] Fantus, B., *J. Am. Med. Assn.*, 1937, **109**, 128.

Over a period of 29-36 days determinations were made of: the plasma proteins using modified micro-Kjeldahl methods; red blood cell fragility; the sedimentation rate of the red blood cells using the Wintrobe tube; the hematocrit value; the total erythrocyte count; the hemoglobin (Newcomer); and the total leucocyte count. The results of these studies are given in Tables I and II. Cultures of the blood at the end of the second and fourth weeks were sterile. Although only 2 specimens of blood were studied, the findings paralleled each other.

There was an appreciable increase in the fragility of the erythrocytes apparent at the fifth day, becoming marked at the end of the third week. This confirms the results recently reported by Belk, Henry, and Rosenstein.[3] The actual number of red cells destroyed

TABLE I.
Observations on Stored Citrated Blood.
Specimen 1.

Day	1	3	5	8	15	22	29	36
Albumin %	3.7	3.9	3.9	3.9	3.6	4.1	5.9	5.2
Globulin %	1.5	1.4	1.2	1.3	2.0	1.8	2.0	3.6
Fibrinogen %	0.195		0.17	0.16	0.16	0.25	0.34	0.39
Fragility (% NaCl)	.46-.30	.46-.32	.50-.32	.50-.34	.54-.36	.85-.38	.85-.38	
Sedimentation rate (mm/hr)	4	1	1	1	0	0	0	
Hematocrit %	40	41	41	41	42	41	38	
Red blood count (millions)	4.2	4.0	4.0	4.0	4.4	4.3	4.0	
Hemoglobin %	76	75	75	79	75	74	78	
White blood count (thousands)	8.9	7.4	8.0	7.2	6.4	4.0	3.3	

TABLE II.
Observations on Stored Citrated Blood.
Specimen 2.

Day	1	3	5	8	15	22	29	36
Albumin %	4.2	3.5	3.7	3.4	3.6	4.4	6.0	6.1
Globulin %	2.1	2.1	2.1	2.0	1.8	1.6	2.6	3.3
Fibrinogen %	0.26		0.28	0.29	0.25	0.36	0.43	0.55
Fragility (% NaCl)	.46-.30	.46-.32	.50-.32	.50-.34	.54-.36	.85-.38	.85-.38	
Sedimentation rate (mm/hr)	8	3	2	3	0	0	0	
Hematocrit %	42	42	42	42	45	44	41	
Red blood count (millions)	4.2	4.0	4.1	4.2	4.3	4.2	4.1	
Hemoglobin %	74	78	78	80	80	78	76	
White blood count (thousands)	9.8	8.5	8.1	7.7	5.8	3.2	2.5	

must have been small, for the total red cell count and hematocrit determinations did not show significant changes. The appearance of hemoglobin in the plasma, which is probably associated with this increased fragility, was first grossly apparent on the fifth day.[4] The total leucocyte count showed a decrease at the end of the second week which became more marked by the third and fourth weeks.[3, 4]

Changes in the concentration of proteins other than hemoglobin in the plasma have not, as far as the authors can determine, been reported previously. There was a significant increase in all of the protein fractions. The actual change in concentration was approximately the same for both albumin and globulin, but the percentage increase in globulin was greater. The change in globulin could not be explained by the hemoglobin content of the plasma, for there was only a trace of visible color at the end of the experiment.

It is interesting to note that not only did the proteins classified as "albumins" and "globulins" increase during the storage of the blood, but that portion described as "fibrinogen" also showed an increase. The nature of this compound cannot be decided definitely, but the increase of the insoluble nitrogen content of the clot formed on the addition of calcium chloride to give a total concentration of less than 0.1% is unquestioned.

It is worth noting that in spite of the changes in the albumin-globulin ratio noted in the first specimen and the increase in apparent fibrinogen observed in both experiments, the sedimentation rate of the erythrocytes did not increase during the period of observation. Instead, there was a significant decrease in the rate from that observed when the specimens were first obtained. The rate became unmeasurable by the method used after the blood had been stored for 2 weeks. The authors were unable to offer any explanation for this unexpected finding, which has apparently not been described.

In addition to the changes in the chemical properties of stored "bank" blood which have previously been reported, an increase in the precipitable nitrogen in the supernatant plasma can be demonstrated. This change is shown by the 3 protein fractions, albumin, globulin, and fibrinogen, after the blood has been preserved for one month. The change is too great to be attributed to the amount of hemoglobin which is present in the plasma. A significant decrease in the rate of sedimentation of the erythrocytes also developed during the period of observation.

---

[3] Belk, W. P., Henry, N. W., and Rosenstein, F., *Am. J. Med. Sci.*, 1939, **198**, 631.

[4] Kolmer, J. A., *Am. J. Med. Sci.*, 1939, **197**, 442.

11167

An Economical Method of Obtaining Blood from the Canary.

HARRY BECKMAN AND ROBERT K. OTA.

From the Department of Pharmacology, Marquette University School of Medicine.

In the ordinarily employed method of making smears of the peripheral blood of the canary in avian malaria studies experience has taught that bleeding from the punctured toe vein is controlled more effectively by releasing the bird at once after obtaining the required amount of blood than by the application of pressure or the use of styptic agents. But the blood loss nevertheless is quite considerable, and when daily smears are being made the anemia which is quickly induced very seriously complicates certain types of study. The object of the present note is to describe a bleeding method in which no more blood is lost than the very small amount actually required for the smear; we have been employing it routinely with entire satisfaction for the past year.

In Fig. 1 it will be seen that the bird is held in the palm of the left hand and that a claw is held in position against the first finger by the thumb of that hand and the tip of the third finger of the right hand while the lancet is poised to make a stab incision at right angles

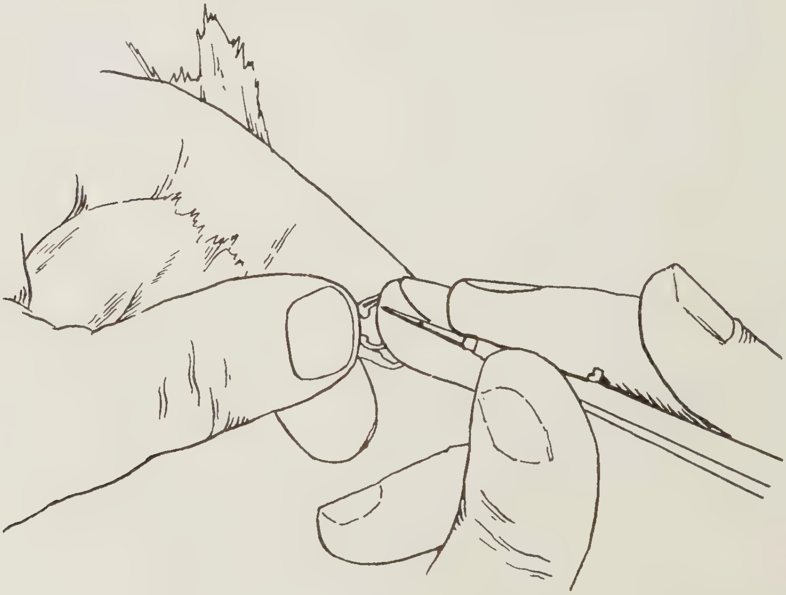


FIG. 1.

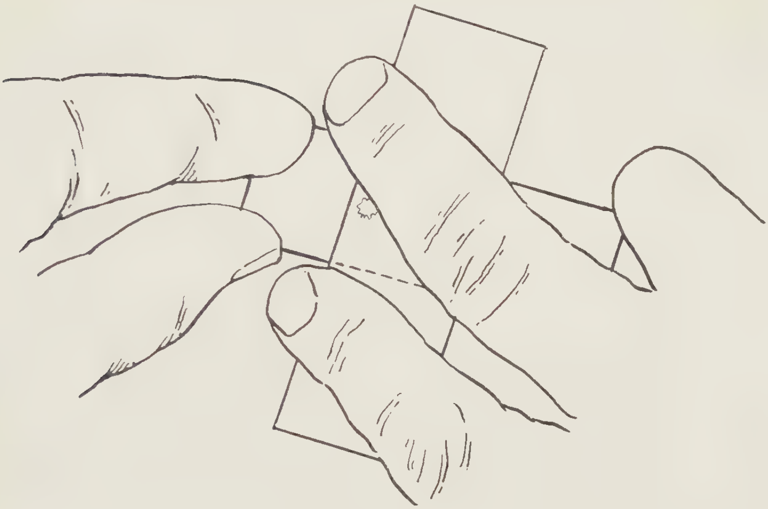


Fig. 2.

to the course of the vessel. Upon appearance of a drop of blood as large as an ordinary pinhead a slide is touched to it and the thumb is at once advanced to cover the wound and stop the bleeding by the momentary application of slight pressure. We have never seen one of these wounds continue bleeding after this manipulation nor have we seen one begin to bleed again when the bird resumes activity; on the other hand, it is often possible to obtain several smears on successive days by lightly applying the lancet to the same incision.

The making of a satisfactory smear from such a small quantity of blood we have found to be somewhat difficult when the classical method of shoving forward with the edge of a second slide held at 45 degrees is employed. But with the method illustrated in Fig. 2 we have excellent results. Just before the blood has ceased to spread between the 2 slides the fingers of the right hand make slight pressure upon the edge of the top slide and it is pulled across to the right with a rather sharp movement.

11168

Effect of Estrogens and Androgens on Growth of Mammary Fibroma in Rats.*

FREDERIC E. MOHS. (Introduced by R. K. Meyer.)

From the Department of Zoology and School of Medicine, University of Wisconsin, Madison.

That the growth of adenofibroma of the mammary gland of rats is restricted in the absence of the ovaries and that this restriction is prevented by estrogen administration has been demonstrated by Heiman and Krehbiel[1] and by Mohs.[2] However, with pure fibroma of the mammary gland no such effect has been previously reported.

This paper concerns a transplantable mammary fibroma, the growth of which is strongly affected by variations in the estrogen level in the body of the host. Occurring spontaneously in the mammary gland of an old female rat this pure fibroma has survived 10 transplant generations over a period of 3 years.

To test the effect of variations in the estrogenic and androgenic hormone levels the tumor was implanted subcutaneously in the inguinal region of rats of 6 different groups as follows: (1) females, (2) castrate females, (3) estrogen-injected castrate females, (4) males, (5) castrate males, and (6) estrogen-injected castrate males. Castration was done 2 weeks before tumor implantation. The estrogen used was Amniotin† in oil and it was injected in 6 rat-unit doses every other day starting 2 days before tumor implantation and continuing to the end of the experiment 4 to 6 months later. At 2-week intervals the tumors were measured with calipers and the mean of the greatest and smallest diameters recorded.

The results (Table I) show that both the success of implantation and the rate of growth of the successful implants were definitely greater in the "estrogen-present" groups (normal females and estrogen-injected castrates) than in the "estrogen-absent" groups (normal males and untreated castrates) the ratio being 2.5:1 in regard to success of implantation and 3:1 in regard to rate of growth.

The finding that in normal males fibroma behaves the same as in castrates of both sexes indicates that the testes have little or no effect

---

* This investigation was aided by the Jonathan Bowman Fund for Cancer Research and by the Thomas E. Brittingham Trust Fund.

[1] Heiman, J., and Krehbiel, O. F., *Am. J. Cancer*, 1936, **27**, 450.

[2] Mohs, F. E., to be published in *Am. J. Cancer*.

† Amniotin was kindly furnished by Dr. John Anderson of E. R. Squibb and Sons.

TABLE I.
Effect of Estrogenic and Androgenic Hormones on Transplantability and Growth Rate of a Pure Fibroma of the Mammary Gland of the Rat.

Hormonal status	No. implanted	Positive No.	Positive %	Growth rate mm/week
Estrogen present				
Normal female	89	38	42.7	3.81
Estrogen-injected castrate female	30	13	43.3	2.14
" " " male	32	19	59.3	3.24
Estrogen absent				
Normal male	59	11	18.6	1.89
Castrate female	41	9	21.9	1.83
" male	35	6	17.1	1.53

on the transplantation or growth of this tumor. However, in 9 castrate females and in 10 castrate males given 250 gamma of testosterone propionate‡ biweekly for 4-5 months this fibroma grew successfully in 44.4% and 90% of the rats respectively. Though the number of testosterone-injected animals is insufficient to justify definite conclusions the data show that, if anything, androgens enhance fibroma growth. This conforms with the findings of Selye, *et al.*,[3] and others that androgens stimulate rather than retard mammary growth in rats.

The greater transplantability and greater growth rate of fibroma in the estrogen-injected castrate males as compared to the estrogen-injected castrate females (59.3% cf. 43.3%) parallels a similar finding previously reported for adenofibroma. It has been tentatively suggested on the basis of work in progress that this phenomenon is due to the greater ease of liberation of mammogenic hormone from the male anterior pituitary as compared to the female.

Malignant connective tissue tumors of the mammary gland, however, lose the ability to respond to hormones. Thus in 2 sarcomas arising in the midst of transplanted adenofibromas no sex difference was found upon transplantation (62.8% of 43 females grew the sarcomas while 65.6% of 64 males had positive implants).

The responses of mammary fibroma to hormonal influences are essentially the same as have been previously demonstrated for benign mammary adenofibroma. In view of the close relationship which the benign tumors of the mammary gland proper have been shown to bear to one another[4] it is probable that all will respond similarly to hormonal effects. Being benign these tumors retain the capacity to respond to the hormones which affect the normal mammary gland.

---

‡ Testosterone propionate was supplied by Dr. Erwin Schwenk of the Schering Corporation.

[3] Selye, H., McEuen, C. S., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 201.

[4] Mohs, F. E., Proc. III Internat. Cancer Cong., Atlantic City, September, 1939.

Summary. The transplantability and rate of growth of a pure mammary fibroma were markedly enhanced by the presence of physiological levels of estrogens in the host rats. Physiological levels of androgens, however, had no demonstrable effect on this tumor. The tumor-stimulating effect of estrogen injections was greater in male castrates than in female castrates. With the onset of malignancy the connective tissue tumors lose their responsiveness to hormonal influences.

11169 P

Destructive Effects on Carcinoma of Colchicine Followed by Distilled Water.*

M. F. GUYER AND P. E. CLAUS.

From the Department of Zoology, University of Wisconsin, Madison.

While studying the enhanced effects of X-rays on transplanted Flexner-Jobling carcinoma following injections of colchicine into the host (paper in press), distilled water injected into the tumors some 15 hours after administration of the drug was used as a control for comparison with the effects of the irradiation. The results are summarized in Table I. Since colchicine alone has a retardative but only occasionally a lethal effect on some tumors[1] it is evident from the tabulation that the subsequent treatment with distilled water was unexpectedly destructive. F.-J. tumors injected with distilled water alone, in 47 rats, showed approximately the same percentage of recoveries as the spontaneous regressions and recoveries in our present strain of rats when the implants are left to grow for 3 weeks and only those regarded as "takes" which have increased to at least 1 cm in diameter; namely, between 3 and 4% (6 of 157 rats). Such 3-week growths were the only kind used in the present experi-

---

* This investigation was aided by support from the Brittingham Trust Fund and a Research Grant from Elizabeth Hopkins Johnson.

[1] Ludford, R. J., *Arch. f. exp. Zellforsch.*, 1936, **18**, 411; Clearkin, P. A., *J. Path. and Bact.*, 1937, **44**, 469; Lits, F. J., Kirschbaum, A., and Strong, L. C., *Am. J. Cancer*, 1938, **34**, 196; Guyer, M. F., and Claus, P. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 565.

TABLE I.

Experiments with Colchicine and Distilled Water.

Tumors all actively growing and ranging in diameter from 1 cm to 2.5 cm; colchicine dosage, 0.1 mg per 100 g of body weight; distilled water injected into tumors 15 hours after subcutaneous injection of colchicine.

Series	No. of rats	Time, end of	Healed	Dead	Unhealed
22	13	7th wk	10	3	0
23	15	12th wk	8	6	1
24	17	10th wk	10	3	4
27	58	8th wk	31	14	13

ments. If one takes beginning growths at the end of only 2 weeks as a criterion, then the spontaneous recovery rate is about 8% (actually 24 of 312 rats).

Rats bearing the rapidly growing cancers were injected subcutaneously with 0.1 mg of colchicine per 100 g of body weight. Fifteen hours later distilled water was injected directly into each tumor in as great quantity as it would hold. By the end of a week the surfaces of most of the growths had sloughed off and many of the cancers appeared to be healing. Some healed entirely after one treatment. Hosts bearing tumors which showed evidence of renewed growth at the end of 2 weeks were treated again as at first. This was repeated at 2-week intervals in all cases showing recurrence of tumor growth. In the 4 series of experiments (see Table I) a total of 103 cancer-bearing animals were used. Complete healing was secured in 59 cases (57.3%). At the conclusion of the experiments 18 tumors (17.5%) remained unhealed and showed no evidence of healing. Of the 26 animals which died, death in several cases, since it followed the water treatment within a few minutes, was due presumably to embolism resulting from injection of the tumors under sufficient pressure to force necrotic tissue into the blood stream. Inasmuch as the cancers were not advanced far enough to be lethal, the remaining deaths, lacking other explanation, were attributed to the unfavorable, warm-weather conditions which prevailed during the course of the experiments.

Just what happens to cancer cells under such treatment is not clear. That it is not merely a matter of pressure is indicated by the fact that similar tumors in rats not given a preliminary treatment of colchicine show no such degree of regression and disappearance following like injections of distilled water. The general histological and cytological picture seen in sections of carcinomas taken from rats 15 to 18 hours after subcutaneous injections of colchicine is strikingly like that observed in similar cancer cells following irradiation. The chromatin of the nuclear contents often becomes clumped into dense masses which stain deeply with basic dyes. This may mean increased acidity and consequently an increased hydrophylic tendency on the part of the cell, particularly the nucleus. It is probable, however, that the cytoplasm is also involved inasmuch as certain of its constituents, namely the Golgi apparatus and the mitochondria are markedly modified within 5 hours after administration of the drug. Under such treatment the Golgi apparatus which ordinarily occurs in the carcinoma cell as a basket-like or looser thread-like mass at one side of the nucleus is dispersed into a cloud of fine droplets, and the usually thread-like mitochondria break up into small rods or granules. Whether the plasma membrane of the cell becomes more permeable to water is not yet known.

There is even noticeable change after some hours in the gross appearance of carcinomas in rats which have been given as high a dosage of colchicine as 0.2 mg per 100 g of body weight. The usual white, waxy appearance of the tumor is replaced by a decidedly reddish color, indicating considerable extravasation of blood. Experiments with various aqueous agents and with other types of tumor are in progress.

Summary. Following administration of colchicine to their hosts 15 hours earlier, Flexner-Jobling carcinomas were found in a majority of 103 cases to be rapidly destroyed by distilled water injected directly into their substance.

11170

A Note upon the Micro-Determination of Ammonia.*

M. F. MASON AND J. D. ROZZELL. (Introduced by C. S. Robinson.)
From the Department of Biochemistry, Vanderbilt University School of Medicine, Nashville.

Several excellent procedures are available for the micro-estimation of ammonia of which the methods of Conway and Byrne[1] and Gibbs and Kirk[2] are notable examples. In connection with studies of tissue respiration it became desirable to determine quantities of ammonia of the order of 5 γ to 50 γ present in the Ringer fluid in which various tissues were suspended. Attempts were made to Nesslerize the deproteinized fluid with measurement of the resulting light transmission with the Evelyn photoelectric colorimeter (Filter 440) as in the micro-non-protein nitrogen determination adapted for this instrument.[3] These were frustrated by immediate clouding of the Nesslerized solutions as a result of the small amount of magnesium ion present in the Ringer fluid[4] employed. Inhibition of the precipitation was not obtained by addition of various stabilizers, *e. g.*, gum ghatti, gum acacia, etc., although the onset and rate of clouding was slightly delayed by adding a small amount of 50% Rochelle salt solution prior to Nesslerization. This slight delay sufficed to make the photoelectric determination feasible. The following technic was found to yield moderately accurate results in spite of interfering magnesium salts.

---

* Aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

[1] Conway, E. J., and Byrne, A., *Biochem. J.*, 1933, **27**, 419.

[2] Gibbs, G. E., and Kirk, P. L., *Mikrochemie*, 1935, **16**, 25.

The fluid in the Barcroft vessel is transferred to a graduated 15 cc centrifuge tube by pipette transfer and the chamber washed 4 successive times with small amounts of distilled water (rendered NH_3-free by treatment with bromine), the washings, in turn, being transferred to the centrifuge tube, making the total volume about 10 cc. This fluid is ordinarily acidic, respiration having been stopped with appropriate quantities of strong acid. If this is not the case, 0.3 cc of NH_3-free 3N H_2SO_4 is added to the contents of the centrifuge tube. This is followed by the addition of 0.22 cc 10% sodium tungstate and 0.11 cc of a 50% solution of Rochelle salts, after which the volume is made to 11 cc with NH_3-free distilled water, and the contents well mixed by agitation with a clean glass rod. Precipitation of the protein is complete in 10 to 20 minutes, after which a clear supernatant fluid may be obtained by centrifugation. Ten cc of this is measured into an Evelyn photoelectric colorimeter tube. A "blank" tube is prepared, containing 1.36 cc of Ringer fluid (which has been made NH_3-free by treatment with bromine), 0.27 cc 3N H_2SO_4, or the corresponding quantity of acid used in stopping respiration, 0.2 cc 10% sodium tungstate, 0.1 cc 50% Rochelle salts, and NH_3-free water to make the final volume 10 cc. Filter 440 is now placed in the colorimeter, and the galvanometer adjusted to the expected center setting (*i. e.*, the reading which is to be obtained following withdrawal of the Nesslerized blank tube when the reading with the latter in place is 100). Two cc of Nessler's reagent are now added to the blank tube, mixed by tapping, and the tube placed in the instrument *immediately*. The galvanometer is quickly adjusted to 100, the tube withdrawn and the immediate center setting value recorded. The whole operation should not occupy over 5 to 7 seconds. With the empty instrument adjusted to the proper center setting reading, the "unknown" tube is Nesslerized similarly, and the immediate galvanometer reading taken, ignoring the slow drift which subsequently occurs.

---

[3] *Evelyn Photoelectric Colorimeter; Notes on Operation*, p. 25, Rubicon Company, Philadelphia, Pa. 1939.

[4] Krebs, H. A., and Henseleit, K., *Z. Physiol. Chem.*, 1932, **210**, 36.

The "K" value is not sufficiently constant over a range of 5 γ to 50 γ to calculate the concentration as a function of the transmission from the Beer-Lambert law, hence a calibration curve is prepared by Nesslerizing tubes containing the reagents present in the blank tube plus graded amounts of a standard ammonium sulfate solution, plotting the *immediate* galvanometer reading against concentration. The concentration of ammonia in an "unknown" tube may then be read off the curve once the galvanometer reading is obtained. Multiplication of this by the factor 1.1 gives the total ammonia content of the fluid in the Barcroft vessel.

Table I shows the recovery of added quantities of ammonia from a series of Ringer fluids in which kidney cortex tissue slices have respired. In our experience, over the range of 10 γ to 50 γ the accuracy of the method is of the order ±5%. For quantities below 10 γ the inherent errors in photolometric measurement considerably decrease the accuracy of the determination, which at concentrations of 5 γ is not better than ±10%.

Although the reaction of Nessler's reagent with ammonia is a very rapid one, it is not entirely completed in the time interval before the galvanometer reading is made. Standardization of technic, in terms of the time intervals employed, eliminates this error by cancellation in the preparation of the calibration curve.

The advantages of this procedure include rapidity, the independence from dilute standard solutions, diminished risk of contamination, as well as the obviating of interference by magnesium salts.

TABLE I.

Recovery of Ammonia Added to Ringer Fluid in Barcroft Vessels; Averages of Duplicate Determinations.

γ NH_3 added	γ NH_3 recovered	% recovery
5.0	4.6	92
10.0	9.5	95
10.0	10.6	106
20.0	20.1	101
30.0	30.7	102
40.0	39.7	99
50.0	50.6	101

11171

Effect of Hyperpyrexia on Tonus of Human Common Bile Duct and Cardiac Sphincters.

Henry Doubilet and William Bierman.

From the Surgical Service of Dr. Ralph Colp and the Department of Physical Therapy, Mount Sinai Hospital, New York City.

It has been known clinically that heat applied to various parts of the body causes muscular relaxation. There are, however, no records in literature to prove that heat actually relaxes intestinal sphincters. Advantage was taken of newly developed methods to record kymographically the effect of hyperpyrexia on the common bile duct sphincter and on the cardiac sphincter.

Methods. A duodenal tube was passed in a patient in whom the common bile duct had been drained by T-tube 2-3 weeks previously. By means of a simple apparatus,[1] a continuous flow of saline was allowed to pass into the T-tube, through the common duct, and into the duodenum. The duodenal tube then drained off the fluid by siphonage. The resistance produced by the sphincter of Oddi could then be measured by means of a water manometer and recorded kymographically on a smoked drum. After obtaining the normal level of resistance of the sphincter, the patient's temperature was raised in a cabinet to approximately 103°F. Changes in the tonus of this sphincter could then be recorded continuously.

Changes in the tonus of the cardiac sphincter were measured by means of a Levine tube to the end of which a balloon was attached. The Levine tube was passed through the cardiac sphincter in a patient who was being treated for cardiospasm. Under fluoroscopic control the balloon was inflated at the point at which the cardiac sphincter compressed its midportion. The inflation was such as to support a column of water in a manometer to the height of 80 mm of water. Changes in the tonus of the sphincter were measured by the rise and fall of this column of water and recorded continuously on a kymograph. Relaxation of the sphincter increased the volume of the balloon and caused the manometric level to fall.

Results. Fig. 1 is a typical record of a tracing showing changes in the tonus of a common bile duct sphincter. Under the influence of rising temperature it can be clearly seen that as the temperature was raised, the tonus of the sphincter gradually decreased from a level of 175 mm to about 125 mm. As the patient cooled, there was only a

---

[1] Doubilet, H., and Colp, R., *Surg., Gynec. and Obstet.*, 1937, **64**, 622.

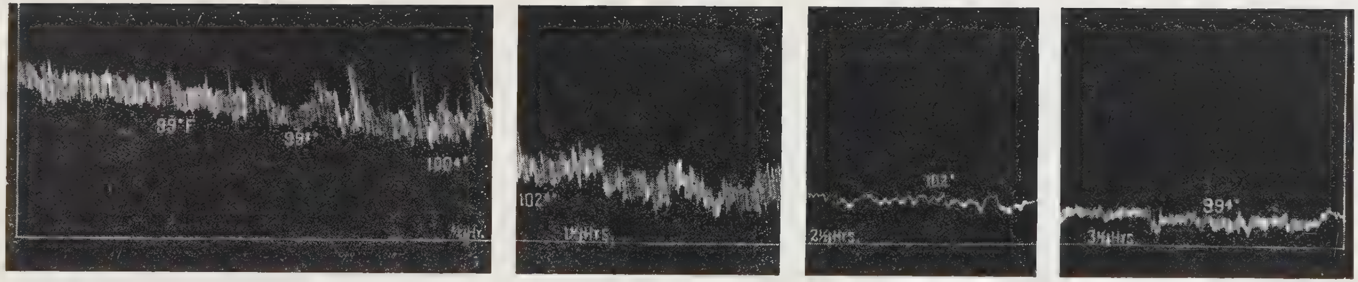


FIG. 1.

Portions of a continuous kymographic record of the resistance of the human common bile duct sphincter under the influence of hyperpyrexia. Note that the resistance of the sphincter fell from a level of 175 mm of water to a level of 125 mm of water after 2 hr of heat.

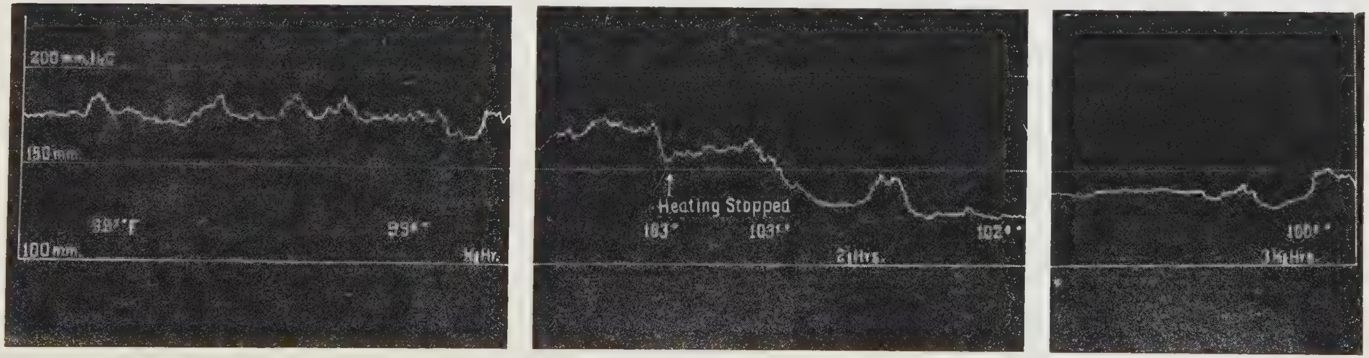


FIG. 2.

Portions of a continuous kymographic record of variations in the tonus of the cardiac sphincter. The fall to the level of the base line indicates that the sphincter is relaxing markedly. The fine tonus rhythm of the cardiac sphincter can be observed at a 2½-hr period very clearly.

slight increase in the tonus which was still low at the end of a 3½-hr. period. Fig. 2 is a typical record showing the decrease in the tonus of the cardiac sphincter in patient who was being treated for cardiospasm by means of hyperpyrexia.

Summary. Kymographic tracings are shown which clearly indicate that hyperpyrexia causes relaxation of the common bile duct sphincter and of the cardiac sphincter in the human.

11172

Bisulfite Binding Substances (B.B.S.) in the Blood and Cerebrospinal Fluid.*

HERMAN WORTIS, ERNEST BUEDING AND WILLIAM E. WILSON.
(Introduced by N. Jolliffe.)

From the Psychiatric Division, the Medical Service of the Psychiatric Division, Bellevue Hospital, New York City, and the Department of Psychiatry, New York University College of Medicine.

The bisulfite binding substances (total aldehydes and ketones) have been the subject of much recent investigation, and their relationship to Vitamin B_1 deficiency has been both confirmed[1] and denied.[2]

The present study of B.B.S. in the blood and cerebrospinal fluid was undertaken in order (1) to compare the levels of bisulfite binding substances in the blood and cerebrospinal fluid, (2) to study the level of bisulfite binding substances in the blood and cerebrospinal fluid in various neuropsychiatric disorders, (3) to determine the relationship of any increase in these carbonyl compounds (B.B.S.) to clinical evidences of Vitamin B_1 deficiency.

Method. Blood and spinal fluid were taken from patients fasting and at rest and delivered into bottles containing dried potassium oxalate. In almost every case, duplicate samples were analyzed without

---

* This work was aided by a grant from Child Neurology Research (Friedsam Foundation).

[1] Sherman, W. C., and Elvehjem, C. H., *Am. J. Physiol.*, 1936, **117**, 142; Sherman, W. C., and Elvehjem, C. H., *J. Nutrition*, 1936, **12**, 321; Thompson, R. H. S., and Johnson, R. E., *Biochem. J.*, 1935, **29**, 694; Johnson, R. E., *Biochem. J.*, 1936, **30**, 31; Platt, B. S., and Lu, G. G., *Proc. Phys. Soc. Third Gen. Congress Chinese Med. Assn.*, 1935; Wilson, H. E. C., and Ghosh, B. C., *Indian Med. Gaz.*, 1937, **72**, 47.

[2] Wilkins, R. W., Taylor, F. H. L., and Weiss, S., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 584; Jong, S., *Arch. Neerland Phys.*, 1936, **21**, 465.

delay. The following modification of the method of Clift and Cook[3] was used.

Ten ml of oxalated blood (or spinal fluid) is precipitated immediately with 40 ml of 10% trichloracetic acid, allowed to stand for 30 minutes and then filtered. Thirty ml of the blood filtrate and 40 ml of the spinal fluid filtrate are adjusted to pH 2 by the addition of 10% NaOH. To this mixture is added 1 ml of molar sodium bisulfite solution and the sample allowed to stand for 15 minutes. Five drops of a freshly prepared 1% starch solution are then added and the excess bisulfite titrated against N/10 iodine solution. The end point is adjusted with N/200 iodine and N/200 sodium thiosulfate solutions and fixed to a one-drop iodine excess. The bound bisulfite is now released by supersaturation with dry sodium bicarbonate salt and the released bisulfite then titrated immediately with an excess of N/200 I_2 solution. This excess is readjusted by titration with N/200 thiosulfate. The rapid addition of I_2 prevents the volatilization and loss of liberated bisulfite. Throughout the titration procedure, the flask is rotated in a medium of cracked ice. A reagent blank is titrated on each occasion.

Calculation. The amount of N/200 I_2 corresponding to the liberated bisulfite is equivalent to the total I_2 minus the thiosulfate. The value of the blank is then deducted from this figure. This difference is then multiplied by 3.67 for the blood sample and 2.75 for the spinal fluid sample. The result obtained equals the total B.B.S. expressed as mg of pyruvic acid per 100 cc.

Results. A comparison of blood and cerebrospinal fluid was obtained on 126 patients with various neuropsychiatric disorders. Wilkins and his coworkers,[1] using a similar technic, reported the normal range for blood as 3.66-5.75 mg %, with an average of 4.74 mg per 100 ml. Platt and Lu,[1] working in the Orient, had previously reported as normal 2.22-4.82 mg %. Since our own control cases (50) fall within the range set up by Wilkins and his coworkers, it was decided to use 5.75 mg % as the upper limit of normal. For purposes of analysis, our cases are divided into 4 groups dependent upon the blood level. Those patients with blood levels above 5.75 mg % were considered abnormally high. (Table I.)

Of these cases 105 had normal ranges for B.B.S. in the blood (2.00-5.72). Their spinal fluid figures ranged from 0.42-3.07 with an average of 1.64 mg %. Only 5 cases were in the range of 0.42-1.00 mg % and most of the cases above 2.25 were inebriates with definite indications of disturbances in vitamin and carbohydrate

---

[3] Clift, F. P., and Cook, R. P., *Biochem. J.*, 1932, **26**, 1788.

TABLE I.

Group	Blood range B.B.S. in mg %	Cerebrospinal fluid range B.B.S. in mg %	C.S.F./Blood, avg %	Standard deviation	No. of cases
I	5.93-13.5 (Avg 7.59)	1.02-8.68 (Avg 3.73)	49%	± 6	21
II.	5.03-5.72 (Avg 5.34)	1.32-2.67 (Avg 2.02)	46%	± 5	8
III.	3.70-4.98 (Avg 4.26)	1.01-3.07 (Avg 1.81)	37%	± 3	51
IV.	2.00-3.63 (Avg 3.13)	0.42-2.36 (Avg 1.37)	44%	± 4	46
				Total	126

metabolism. Children apparently had somewhat lower figures in blood and cerebrospinal fluid than adults, but the results were well within the normal range.

The material presented consists of 126 cases from the wards at the Psychiatric Division of Bellevue Hospital. B.B.S. levels were determined in the blood and cerebrospinal fluid. They formed the following diagnostically labelled groups:

Group		No. of cases
I.	Inebriates	10
II.	Inebriates with paraldehyde therapy	10
III.	Delirium tremens	7
IV.	Alcoholic hallucinosis	4
V.	Wernicke's syndrome	4
VI.	Korsakoff syndrome	2
VII.	Central nervous system syphilis	12
VIII.	Schizophrenia—all types	25
IX.	Manic-depressive psychosis	4
X.	Miscellaneous psychoses and neuroses	11
XI.	Miscellaneous medical conditions	7
XII.	Behavior and conduct disturbances in children	30†

Our results indicated the following: 1. The normal range of B.B.S. in the spinal fluid is 0.42-3.07 mg %. The average for 106 cases with blood below 5.75 is 1.64 mg % with a standard deviation of ±0.06, 91 fell within a range of 1.00-2.25 mg %, which is usually 35-60% of the amount found in a simultaneous blood sample.

2. The B.B.S. may or may not be elevated in cases of clinical Vitamin B_1 deficiency (peripheral neuropathy, beri-beri—possibly Korsakoff and Wernicke's syndromes) but this is also true for conditions in which there is no clinical evidence of Vitamin B_1 deficiency. Eight cases of peripheral neuropathy in inebriates were studied. This syndrome in inebriates is the result of Vitamin B_1

---

† Of the total 126 cases, 8 cases had peripheral neuropathy as a complicating syndrome (3 in Group 1, 3 in Group III, and 2 in Group V).

deficiency.[4] Four cases showed an elevated B.B.S. in the blood or cerebrospinal fluid or both, and the other 4 had normal figures. These variations obtained in both acute and chronic cases. Hence the B.B.S. in blood and cerebrospinal fluid cannot be used as an indication of Vitamin B_1 deficiency.

3. A comparatively large group of psychiatric disorders including alcoholic psychoses, schizophrenia, manic-depressive psychoses, miscellaneous psychoses, and behavior and conduct disorders in children were studied. No constant deviation from the normal was found.

4. Paraldehyde causes an elevation of B.B.S. in the blood and cerebrospinal fluid for 24 hours. The drug itself does not bind bisulfite, but our experiments show that the method employed (precipitation with trichloracetic acid) causes a partial hydrolysis of paraldehyde to acetyldehyde, which latter binds bisulfite. Furthermore, patients given paraldehyde, drams 2, by mouth or rectum showed a marked rise in blood and spinal fluid B.B.S. for at least 24 hours. The effect of sodium amytal is under investigation.

5. Our unpublished observations indicate that the B.B.S. is not an accurate measure of the pyruvic acid levels in the blood or cerebrospinal fluid. Several cases of peripheral neuropathy in inebriates were observed with elevated pyruvic acid levels in the body fluids, and a normal total B.B.S.

11173

Potassium as a Toxic Factor in Intestinal Obstruction.

Myron N. Jorgensen, Nicholas Dietz and Frederick C. Hill.
From the Department of Biochemistry and the Department of Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska.

A satisfactory explanation of the cause of death in intestinal obstruction is not apparent at the present time. Many hypotheses have been advanced[1–4] and of these the view that potassium is a toxic factor, has received attention of late. Scudder, Zwemer and Whipple[5] attributed the earlier development of toxemia in cases of intestinal strangulation and gangrene "to a more rapid and sustained rise in blood potassium, occasioned by loss of fluids."

---

[4] Jolliffe, N., and Colbert, C. N., *J. A. M. A.*, 1936, **107**, 642.

[1] Gamble, J. L., and Ross, S. G., *J. Clin. Invest.*, 1925, **1**, 403.

[2] Hartwell, J. A., and Houget, J. P., *J. Am. Med. Assn.*, 1912, **59**, 82.

[3] Knight, G. C., and Slome, D., *Brit. J. Surg.*, 1936, **23**, 820.

[4] Gatch, W. D., and Culbertson, C. G., *Ann. Surg.*, 1935, **102**, 619; Whipple, G. H., Stone, H. B., and Bernheim, B. M., *J. Exp. Med.*, 1913, **17**, 307; Dragstedt, L. R., Dragstedt, C. A., McClintock, J. T., and Chase, C. S., *J. Exp. Med.*, 1919, **30**, 109.

The present report presents data from experiments in which the blood potassium level was measured, before and at intervals throughout the period of survival, of dogs suffering from intestinal obstruction produced by surgical means.

Methods and Technic. All surgical procedures were performed under ether anesthesia by careful aseptic methods and were accomplished quickly and with a minimum of trauma. Obstructions were produced by (1) Dividing the intestine at the ligament of Treitz and suturing the inverted ends. Dogs 1, 2, 4, 5. (2) Tying off the blood supply to a segment of intestine, thereby producing extensive vascular derangement without segmental obstruction. Dogs 11, 12, 13, 14. (3) Tying off a segment of intestine with heavy cord, *i. e.,* producing segmental obstruction plus vascular derangement (mesenteric thrombosis). Dogs 6, 7, 8, 9, 10.

Blood samples were drawn from the jugular vein or femoral artery of the living animals and the samples taken at death were obtained from the right heart. Oxalate was used as the anticoagulant. Plasma was separated by centrifugation and the proteins precipitated with tungstic acid.

Blood potassium was determined by a slight modification of the method of Truskowski and Zwemer.[6, 7] Duplicate determinations, checked by the analysis and recovery of known quantities of potassium, were conducted in all experiments. Blood chlorides and carbon-dioxide-combining capacity were conducted according to the methods of Whitehorn[8] and Van Slyke and Cullen[9] respectively.

Results. The normal blood potassium level of each dog was followed for a period of 6-39 days since it appeared desirable that emphasis should be placed on the change in the blood potassium level rather than on an absolute value. The normal values observed for whole blood and plasma potassium on 15 dogs appear in Table I (upper portion). The range for normal whole blood potassium values varied from 17.6 to 37.2 mg %, with an average of 23.5. The highest value was observed in the pregnant female dog 6. The normal range for plasma potassium values varied from 11.8 to 30.6 mg %, average 20.2.

---

[5] Scudder, J., Zwemer, R. L., and Whipple, A. O., *Ann. Surg.*, 1938, **107**, 161.

[6] Truskowski, R., and Zwemer, R. L., *Biochem. J.*, 1936, **30**, 1353.

[7] Truskowski, R., and Zwemer, R. L., *Biochem. J.*, 1937, **31**, 229.

[8] Whitehorn, J. C., *J. Biol. Chem.*, 1921, **45**, 449.

[9] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1917, **30**, 289.

Following operation, we found the blood potassium values in individual experiments were subject to a greater variation than during the control period, but maximum concentrations observed (Table I, lower portion) did not differ markedly from maximum control values. We found no evidence in these experiments of the "rapid and sustained" rise in blood potassium observed by Scudder, *et al.*, in their series. Indeed, we believe that in many of the cases cited by them, the serum potassium was within normal limits during obstruction. In our experiments on dogs 1, 2, 4, 5, 10, 11, 12, and 13, however, the blood picture was not complicated by the loss of fluid by vomiting or exudation into the intestine or peritoneal cavity.

Autopsies conducted at the time of death revealed the absence of septicaemia, peritonitis or gross pathological conditions other than the following: Perforation of the gangrenous intestine occurred in dog 6. A considerable quantity of a characteristic dark, bloody, foul-smelling exudate was found in the intestine of dogs 6, 8, and 9. Analysis of this fluid indicated the presence of 225, 113, 368 mg % of potassium respectively. A peritoneal exudate found in dog 14 contained 121 mg % of potassium. That transperitoneal absorption of this potassium occurred and was responsible for the death of these animals seems unlikely, however, since only dog 8 showed an increase in blood potassium which might be considered toxic. Fifty mg of KCl per pound of body weight was administered to dog 15 (Table I)

TABLE I.
Blood Potassium Values in Experimental Intestinal Obstruction.
Control Period.

		Whole blood K mg%			Plasma potassium mg%		
Dog No.	Control period, days	Samples	Observed variations	Avg	Samples taken	Observed variations	Avg
1.	18	7	21.0-24.2	23.2	6	19.2-23.6	20.5
2.	19	4	19.5-23.5	21.5	5	20.2-21.6	21.3
3.	6	3	19.2-21.5	20.1	3	18.5-21.3	19.6
4.	32	5	19.1-23.0	21.4	6	13.0-24.9	19.6
5.	14	4	21.2-37.2	31.0	5	17.0-26.0	20.4
6.	34	8	17.7-27.4	21.9	8	12.6-23.1	19.1
7.	17	4	18.3-25.6	20.6	4	15.2-19.6	17.4
8.	22	6	17.8-32.4	22.9	7	14.0-30.2	21.8
9.	19	6	19.6-32.3	23.5	6	16.6-23.2	19.7
10.	18	6	22.6-31.3	26.7	6	16.3-25.4	20.9
11.	39	8	20.4-32.0	23.2	8	14.4-30.6	20.3
12.	28	13	17.6-27.5	22.4	13	11.8-24.6	18.4
13.	34	11	18.0-28.2	22.4	9	13.1-26.1	19.2
14.	35	10	20.7-35.0	26.2	3	19.8-26.8	22.3
15.	26	7	19.9-30.0	26.5	7	19.6-29.5	23.1
Total or avg		147	17.6-37.2	23.5	96	11.8-30.6	20.2

TABLE I. (Continued)
Period of Experimental Obstruction.

				Whole blood K		Plasma potassium	
Dog No.	Type of exp. Obstruction	Survival period	Samples taken	Observed variations	At near death	Observed variations	At near death
1.	Intestine severed below ligament of Treitz. Ends sutured	14½ days	20	16.9-30.2	28.1	9.2-37.6	21.1
2.	*Ibid.*	4½ ”	7	17.8-30.2	23.9	15.5-20.9	15.5
3.	*Ibid.*			Gangrene evident			
4.	*Ibid.*	15½ ”	13	16.1-27.1		12.4-23.2	
5.	*Ibid.* Pregnant female	2½ ”	6	22.3-28.9	35.5	25.6-26.9	49.0
6.	Strangulated intestinal loop 18 in. just below ligament of Treitz	24 hr	3	22.4-23.9	22.4	21.4-23.2	21.4
7.	*Ibid.* 9-in. loop	24 ”	4 hr	17.7-24.6	23.6	13.5-16.2	13.6
8.	*Ibid.*	24 ”	4			22.0-23.2	44.8
9.	*Ibid.*	26 ”	3	27.0-34.1	36.1	21.5-22.5	21.5
10.	*Ibid.* 2-in. loop	36 ”	7	23.7-30.1	35.9	15.4-33.9	15.4
11.	Ligated mesentery blood supply about 12 in. of intestine. Blood vessels ligated	11 ”	5	17.7-28.0	28.0	19.3-19.9	35.3
12.	*Ibid.*	46 ”	9	22.2-30.4	34.6	18.4-32.6	24.1
13.	*Ibid.*	36 ”	11	24.6-31.7	36.0	20.1-34.0	24.9
14.	Blood vessels of mesentery ligated *Ibid.*	48 ”	8	18.2-30.9	25.4	Chlorides varied 527-589 mg%	
15.	Control female pregnant appendectomy. Aborted 3rd day after operation		11	18.5-30.7		11.8-25.7	Survived
	Injection of 1.9255 g KCl (50 mg KCl per lb)		7	20.8-32.6		18.0-26.5	Survived
Total or avg			118	16.1-34.1		9.2-37.6	

subsequent to an appendectomy, which served as a control operation. This dog, a pregnant female deprived of food and drink, showed no toxic symptoms of potassium poisoning; yet the observed changes in the concentration of the blood potassium were not unlike those found in dogs dying from intestinal obstruction.

Dogs 1, 2, 4, 5, 10, 11, 12, and 13 were characterized by the almost total absence of a fluid exudate either in the intestine itself or in the peritoneal cavity, and the dogs did not vomit. Obviously, death cannot be ascribed to loss of fluid in these 8 experiments.

The changes of relatively small magnitude in blood potassium observed in the present study could well be accounted for on the basis of purely mechanical factors such as (1) change in plasma volume, (2) change in resistance or rate of blood flow, or, since one of the earliest evidences of a disturbance in intestinal obstruction is a lessening of urinary output, (3) to anuria. Baetjir[10] noted the interesting fact that potassium escapes from the muscles and elevates the blood potassium when the blood flow through a limb is reduced by hemorrhage, vasoconstriction or clamping of an artery. Baetjir found a marked increase in plasma potassium to occur under these conditions. Similarly, Dennis and Moore[11] observed that ischemia of the functioning heart of the cat led to a marked rise in the potassium content of the venous blood. It is of interet here also, to note that periods of anoxia of 2-4 minutes' duration result in an average increase of blood potassium of 30% in the dog (Dennis and Mullin[12]), or average increase of 120% for 4-5 minute of asphyxia in the cat (Cattell and Civin[13]). The administration of adrenalin (Marenzi and Gerschman,[14] D'Silva,[15] and others); histamine (Thaler[16]), or central nervous stimulation (Cloella, Fisher and van der Loeff[17]) all result in effecting a marked rise in serum potassium. The mechanisms involved in these changes may well account for changes observed in the blood potassium in intestinal obstruction. The changes in blood potassium observed by the above group did not result in apparent damage since all animals survived, although changes in serum, plasma or whole blood potassium values were greater than those observed in the present study. Thus it becomes increasingly difficult to assume that potassium is a toxic factor in intestinal obstruction.

In view of the specific action of potassium on the heart, the observations of Winkler, Hoff and Smith[18] appear to us further to substantiate the view that potassium cannot be considered as contributing to the cause of death in intestinal obstruction. These workers studied the electrocardiographic changes which resulted following a constant elevation of the serum potassium in the dog, produced by intravenous injection of isotonic KCl. Intraventricular block began when the serum potassium reached a concentration of 10mM per liter (39 mg %) and cardiac arrest took place when the critical concentration of 14.16 mM per liter (55-63.5 mg %) was reached. Ectopic arrythmias were not observed to occur and all dogs survived the experiment despite an average increase in potassium of 300%. Furthermore, according to Scudder, Smith and Drew,[19] the plasma potassium of 4 dogs receiving isotonic potassium in lethal doses reached 50.5 mg % before cardiac arrest and death occurred. Blood potassium values of this order were not observed in the present series. We feel that the quantitative changes in the biochemical composition of the blood as observed in intestinal obstruction are incidental to the disturbances in blood volume, concentration, rate of flow and tissue metabolism rather than the cause of the prostration and death.

---

[10] Baetjir, A. M., *Am. J. Physiol.*, 1935, **112**.

[11] Dennis, J., and Moore, R. M., *Am. J. Physiol.*, 1938, **123**, 443.

[12] Dennis, J., and Mullin, F. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 560.

[13] Cattell, M., and Civin, H., *J. Biol. Chem.*, 1938, **126**, 635.

[14] Marenzi, A. D., and Gerschman, R., *Compt. rend. soc. biol.*, 1937, **124**, 382; *Rev. soc. argentia biol.*, 1936, **12**, 424.

[15] D'Silva, J., *J. Physiol.*, 1937, **90**, 303.

[16] Thaler, J. I., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 368.

[17] Cloetta, M., Fisher, H., and van der Loeff, M. K., *Arch. exp. Path. Pharm.*, 1934, **174**, 589.

[18] Winkler, A. W., Hoff, H. E., and Smith, P. K., *Am. J. Physiol.*, 1938, **124**, 478.

Bisgard, McIntyre and Osheroff[20] studied the behavior of serum potassium in the blood of dogs suffering from high intestinal obstruction or duodenal fistulas. They found no evidence that the potassium or a change in the sodium-potassium ratio contributed to the production of symptoms or cause of death. Our results confirm this view. Recently Falconer, Osterberg and Bargen[21] arrived at a similar conclusion as a result of their observations on patients suffering from intestinal obstruction.

Summary. It has been shown that hyperpotassemia cannot be considered as a contributing factor to the cause of collapse and death in experimental intestinal obstruction in the dog.

---

[19] Scudder, J., Smith, M. E., and Drew, C. R., *Am. J. Physiol.*, 1939, **126**, 337.

[20] Bisgard, J. D., McIntyre, A. R., and Osheroff, W., *Surgery*, 1938, **4**, 528.

[21] Falconer, M. A., Osterberg, A. E., and Bargen, J. A., *Proc. Staff Meetings Mayo Clinic*, 1939, **14**, 22.

11174

Response of Different Strains of Type I Pneumococcus to Sulfapyridine.

L. H. Schmidt and Carolyn Hilles. (Introduced by S. Tashiro.)

From the Christ Hospital Research Institute and the Department of Biochemistry, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

Studies on the effectiveness of sulfapyridine therapy in experimental infections with type I pneumococcus have given conflicting results. Some investigators[1-5] have found that sulfapyridine has a marked curative action in such infections, while others[6-9] have reported that the drug has little or no curative action and at best merely prolongs life. It has been suggested[3, 7] that these conflicting observations may have been due to the use of different strains of pneumococcus. Some support for this suggestion has been offered by the *in vitro* experiments of MacLean, Rogers and Fleming,[10] which showed that strains of pneumococcus differed in their sensitivity to sulfapyridine; however, the strength of this support has been weakened by the recent observation of Long and Bliss,[11] that 2 strains of type I pneumococcus which reacted quite differently to sulfapyridine *in vitro,* responded similarly to the drug *in vivo.* Since there has been no extensive study of the variations in sensitivity of different strains *in vivo,* it seemed desirable to determine whether such variations occur, and if so, whether certain strains are completely resistant to sulfapyridine.

The present report concerns a study of the effectiveness of sulfapyridine therapy in experimental infections with 12 strains* of

---

[1] Whitby, L. E. H., *Lancet,* 1938, **1**, 1210.

[2] Schmidt, L. H., and Hilles, C., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 611.

[3] Greey, P. H., MacLaren, D. B., and Lucas, C. C., *Canad. Med. J.,* 1939, **40**, 319.

[4] MacLeod, C. M., and Daddi, G., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 69.

[5] Powell, H. M., and Chen, K. K., *J. Pharm. and Exp. Therap.,* 1939, **67**, 79.

[6] Long, P. H., Bliss, E. A., and Feinstone, W. H., *Penn. Med. J.,* 1939, **42**, 483.

[7] Bliss, E. A., Feinstone, W. H., Garrett, A. W., and Long, P. H., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 619.

[8] Marshall, E. K., personal communication.

[9] Raiziss, G. W., Severac, M., Moetsch, J. C., and Clemence, L. W., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 12.

[10] MacLean, I. H., Rogers, K. B., and Fleming, A., *Lancet,* 1939, **2**, 563.

[11] Long, P. H., and Bliss, E., *Ann. Int. Med.,* 1939, **13**, 232.

* Cultures of pneumococci were obtained through the kindness of the following persons: strains Neufeld, NYS 55, NYS 23 and NYS 63 from Dr. Julia Coffey, New York State Department of Health, Albany; strain Felton from Dr. E. K.

type I pneumococcus—identical experiments being performed with each of the strains except strain Neufeld. Three groups of white mice were infected, intraperitoneally, with approximately 100 lethal doses of a given strain. The mice in one of these groups served as untreated controls. Those in the other groups (A and B) were treated with sulfapyridine according to the schedules† shown in Table I, the drug being administered, orally, as a 10% suspension in 10% acacia. On the tenth day, the surviving mice were divided into 2 groups, one group being kept under observation for an additional 20 days,‡ the other being used in the reinfection experiments described below.

The results of these therapeutic experiments (Table I) show that there were marked quantitative variations in the response of different strains of type I pneumococcus to sulfapyridine. Although these variations were evident in both the Group A and Group B experiments, they were particularly marked in the Group A experiments—those in which the mice received intensive treatment only during the first day and moderate therapy thereafter.§ This treatment was most effective against strain Neufeld, 98% of the mice surviving, and was least effective against strain McGovern, only 23% of the mice surviving. Two of the other strains (M 36 and NYS 55) responded to the drug nearly as well as did strain Neufeld. The remaining 8 strains responded more like strain McGovern, 24 to 43% of the mice surviving infection.

Although moderate sulfapyridine treatment (Group A experiments) was highly effective against infections with strains Neufeld, NYS 55 and M 36, only when intensive treatment was maintained throughout the 6-day period was the drug highly effective against

---

Marshall, Jr., School of Medicine, Johns Hopkins University, Baltimore, Md.; strain M 36 from Dr. M. Severac, Dermatological Research Laboratories, Philadelphia, Pa.; and strains Lederle 6, Bonner, McGovern, Lockhart, Chauncey and Clement from Dr. Raymond Libby, Lederle Laboratories, Inc., Pearl River, N. Y. The technique used in preparing these pneumococci for therapeutic experiments has been described elsewhere.[12]

† These schedules were based on our earlier work[2] which showed that the treatment given to Group A sufficed to cure a majority of mice infected with highly susceptible types of pneumococci; that given to Group B was effective against infections with moderately susceptible types, but only prolonged life in infections with such resistant types as II, III and VIII.

‡ In this group no deaths occurred later than 10 days after infection.

§ Using a slight modification of Marshall's method[13], it was found that the treatment given to Group A mice maintained a minimum level of 16 mg % free sulfapyridine during the first 24 hours of the experiment and a minimum level of 2 mg % thereafter. The treatment given to Group B mice maintained a minimum level of 16 mg % throughout the entire experiment.

TABLE I.

Summary of Therapeutic and Reinfection Experiments.

Strain of Pneumococcus	Therapeutic experiments: Infecting dose				No. of deaths: Days after infection							10-day survivors		Reinfection experiments: Infecting dose			Survivors 20 days after reinfection	
	cc 12-hr cult.	No. of colonies	Group	No. of mice in group	1	2	3	4	5	6	7 to 10	No.	%	cc 12-hr cult.	No. of colonies	No. of mice reinfected	No.	%
Neufeld	10^{-6}	160	Control	20	10	10	0	0	0	0	0	0	0	10^{-6}	130	10*	0	0
			A	50	0	0	0	0	1	0	0	49	98			25	21	84
Felton	10^{-6}	130	Control	40	30	10	0	0	0	0	0	0	0	10^{-7}	60	10*	0	0
			A	70	1	1	1	13	29	7	1	17	24			10	2	20
			B	30	0	0	0	1	0	0	2	27	90			15	3	20
NYS 55	10^{-6}	150	Control	20	5	7	8	0	0	0	0	0	0	10^{-6}	150	10*	0	0
			A	30	0	1	0	0	0	0	11	18	60			10	7	70
			B	30	0	0	0	0	0	0	0	30	100			14	13	93
NYS 63	10^{-6}	1940	Control	20	3	7	10	0	0	0	0	0	0	10^{-6}	1210	10*	0	0
			A	30	0	0	0	0	0	0	20	10	33			5	3	60
			B	30	0	0	0	0	0	3	1	26	87			13	13	100
NYS 23	10^{-6}	110	Control	20	4	8	8	0	0	0	0	0	0	10^{-6}	140	10*	0	0
			A	30	0	0	0	2	0	1	17	10	33			5	3	60
			B	30	0	0	0	6	1	0	2	21	70			10	10	100
M 36	10^{-6}	120	Control	30	25	5	0	0	0	0	0	0	0	10^{-6}	120	10*	0	0
			A	30	0	1	5	2	0	0	0	22	73			10	0	0
			B	50	0	0	0	1	1	0	3	45	90			23	3	13
Chauncey	10^{-5}	890	Control	20	0	13	7	0	0	0	0	0	0	10^{-5}	960	10*	0	0
			A	30	0	0	0	0	0	1	16	13	43			7	3	43
			B	30	0	0	0	1	0	0	4	25	83			13	11	85

Lederle 6	10^{-7}	60	Control	20	9	9	2	0	0	0	0	0	0	10^{-7}	60	10*	0	0
			A	30	0	0	0	1	8	1	11	9	30			5	2	40
			B	30	0	0	0	0	0	0	1	29	97			14	2	14
McGovern	10^{-7}	70	Control	20	15	4	1	0	0	0	0	0	0	10^{-7}	40	10*	0	0
			A	30	0	0	0	11	2	3	7	7	23			3	1	33
			B	30	0	0	0	1	1	2	3	23	77			12	10	83
Bonner	10^{-6}	90	Control	20	5	12	3	0	0	0	0	0	0	10^{-6}	160	10*	0	0
			A	30	0	0	0	0	0	0	18	12	40			6	1	17
			B	30	0	0	0	0	0	0	4	26	87			13	8	62
Lockhart	10^{-6}	300	Control	20	0	16	4	0	0	0	0	0	0	10^{-6}	240	10*	0	0
			A	30	0	0	0	0	1	1	19	9	30			5	3	60
			B	30	0	0	0	1	2	2	1	24	80			10	9	90
Clement	10^{-5}	2300	Control	20	2	14	4	0	0	0	0	0	0					
			A	30	0	0	0	0	2	4	16	8	27					
			B	30	0	0	0	1	0	0	12	17	57					

* New controls—not infected previously.

Group A mice received 20 mg of sulfapyridine 2, 8, 14 and 22 hours after infection and every 24 hours thereafter for 5 days.

Group B mice received 20 mg of sulfapyridine 2, 8, 14 and 22 hours after infection and every 8 hours thereafter for 5 days.

the other 9 strains. Under such intensive treatment§ (Group B experiments) 57 to 100% of the mice survived infection, and in only one experiment—that with strain Clement—was the recovery less than 70%. This observation, that the majority of strains of type I pneumococcus responded well only to prolonged intensive treatment, probably explains some of the earlier conflicting reports on the effectiveness of the drug. The superior results of the Group B experiments, as compared with those of Group A, reëmphasize the importance of administering sulfapyridine at frequent and regularly spaced intervals so as constantly to maintain effective levels of the drug in the blood.[3, 12]

It is evident from the data in Table I that the variations in the response of different strains to sulfapyridine were not related to the size of the infecting dose nor to the virulence of the organisms. It is also apparent that the variations were not related to the invasiveness of the strains, as had been suggested previously,[7] for the drug was equally effective against strains of high and low invasiveness. It occurred to us that the effectiveness of sulfapyridine might be related to the capacities of the strains to stimulate production of antibodies, since the antigenicity of strains of pneumococcus varies considerably and recovery from pneumococcal infections generally depends on antibody production. Such a possibility seemed to be supported by MacLeod's observation[14] that the effectiveness of sulfapyridine against Types I and III pneumococci was proportional to the antigenicity of these types.

In order to test this idea we have determined whether the mice that survived the therapeutic experiments were equally immune to reinfection. Ten days after the initial infection (4 days after termination of treatment) half of the surviving mice from Groups A and B were reinfected with approximately the same number of pneumococci (homologous strain) as in the original experiment—suitable controls being reinfected simultaneously.

The results of these experiments (Table I) show that the immunity of sulfapyridine-treated mice to reinfection depends to a great extent on the strain of pneumococcus used as the infecting agent.‖ Mice infected with 8 of the strains (Neufeld, NYS 55, NYS 63, NYS 23, Chauncey, McGovern, Bonner and Lockhart) were highly

---

[12] Schmidt, L. H., and Hilles, C., *J. Infect. Dis.*, 1939, **65**, 273.

[13] Marshall, E. K., *J. Biol. Chem.*, 1937, **122**, 263.

[14] MacLeod, C. M., *J. A. M. A.*, 1939, **113**, 1405.

‖ This observation provides an explanation for conflicting reports[1, 15, 16] on the immunity of sulfapyridine-treated mice to reinfection.

immune to reinfection, 62 to 100% of the Group B mice surviving. Mice infected with the 3 remaining strains (Felton, M 36 and Lederle 6) had little immunity to reinfection. Only 5 of 25 mice (Groups A and B) reinfected with strain Felton, 3 of 33 reinfected with M 36, and 4 of 19 reinfected with Lederle 6 survived.

A comparison of these results with those of the therapeutic experiments shows that there is little correlation between the effectiveness of sulfapyridine and immunity to reinfection. Thus the drug was equally effective against strains Felton, Lederle 6, McGovern, Bonner, and Lockhart, but mice that survived infection with the first 2 strains had little immunity to reinfection, whereas those surviving infection with strains McGovern, Bonner, and Lockhart were highly immune.

It is evident, therefore, that differences in the antigenicity of the strains were not primarily responsible for the variations in the effectiveness of sulfapyridine.

Since the variations in the response of different strains of type I pneumococcus to sulfapyridine do not appear to be related to such characteristics of the strains as virulence, invasiveness and antigenicity, there must be differences in other characteristics which determine the effectiveness of the drug. The work of Mellon and coworkers[17] suggests that the capacity to produce hydrogen peroxide may be a determining factor, while MacLeod's observations[18] on *"sulfapyridine-fast"* pneumococci suggest that sensitivity to the drug may depend on the manner in which carbohydrate is oxidized. These possibilities are now being investigated.

Summary. (1) Strains of type I pneumococcus varied considerably in their response to sulfapyridine. (2) These variations were not as great as the variations between types, observed previously,[2] for all the strains of type I pneumococcus were susceptible to intensive therapy. (3) Reinfection experiments indicated that there were marked variations in the antigenicity of the strains, but these differences could not be correlated with the effectiveness of sulfapyridine, and therefore were not responsible for the strain variations noted above.

---

[15] Long, P. H., *J. A. M. A.*, 1939, **112**, 538.

[16] Schmidt, L. H., and Hilles, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 111.

[17] Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.

[18] MacLeod, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 215.

11175

Specific Aggregation of Streptococcal Proteins Adsorbed on Oil Droplets.*

DANIEL A. BOROFF. (Introduced by Stuart Mudd.)

From the Department of Bacteriology, University of Pennsylvania School of Medicine, Philadelphia, Pa.

Emulsions of oil droplets in solutions of certain streptococcal proteins have proven to be a means of transforming precipitative reactions into agglutinative reactions. Type-specific aggregation, by means of antistreptococcal antisera, of oil droplets emulsified in solutions of Lancefield's "M" substance are of particular interest.

The solutions used have been solutions of the proteins extracted from *Streptococcus pyogenes* (Group A β-hemolytic streptococci) at neutrality[1] as well as the acid-soluble type-specific protein (Lancefield's "M") from *Streptococcus pyogenes*.[2, 3] The antisera used were rabbit sera prepared by injecting rabbits with living cultures of Type 1 and Type 6 streptococci, and by injecting rabbits with a solution of the neutral extractable proteins.

The experimental technic employed follows:

Preparation of the antigen. 0.5 cc of olive oil was emulsified in 20 cc of distilled water. This emulsification was accomplished by putting the material in the magnetostriction oscillator of Chambers† and Gaines and treating with sound waves.[4, 5] To this emulsion was added the protein solution. This was prepared by dissolving 25 mg of protein in 5 cc of distilled water and carefully adjusting the pH to 7.4 (brom thymol blue indicator). The protein solution and emulsion were then shaken for 15 minutes in a Kahn-test shaker and allowed to stand overnight in the refrigerator. Examination of such an emulsion under the dark field microscope showed many minute droplets of oil homogeneously dispersed in water.

Macroscopic Test. To 0.2 cc of antiserum dilution was added 0.2

---

* The expenses of this work have been defrayed by a generous grant from The Commonwealth Fund.

[1] Sevag, M. G., Lackman, D. B., and Smolens, J., *J. Biol. Chem.*, 1938, **124**, 425.

[2] Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 91.

[3] Zittle, C. A., *J. Immunol.*, 1939, **37**, 1.

† We wish to acknowledge the assistance of Dr. Leslie Chambers in preparing the oil emulsions.

[4] Chambers, L. A., and Gaines, N., *J. Cell. Comp. Physiol.*, 1932, **1**, 465.

[5] Flosdorf, E. W., Chambers, L. A., and Malisoff, W. M., 1936, **58**, 1069.

cc of a 1:3 dilution of the antigen. After thorough shaking, the tubes were placed in the 56°C bath overnight. In the morning the tubes were centrifuged and read. In reading the test it must be borne in mind that agglutinated oil droplets will be found in a creamy layer at the surface of the liquid. The degree of reaction is evaluated by the degree of clearing of the underlying liquid and the compactness of the creamy layer obtained upon centrifuging.

Slide Test. It was found to be possible to set up the above test as a microscopic test. In this test a large drop of antigen and of antiserum, diluted to a suitable concentration, were mixed upon a slide. The reaction was very rapid and striking; if positive, the oil droplets were seen to aggregate into large clumps. This reaction is best observed with a dark field microscope; however it may be observed with the high dry objective and when the reaction has progressed sufficiently, the aggregated oil droplets may be seen with the unaided eye.

A refinement of this test which permits of continued observation and of preservation is as follows: a coverslip was attached to a slide by means of paraffin placed on two edges of the slip; a drop of antigen dilution and of serum dilution were allowed to flow from opposite sides under the coverslip and meet in the center; the coverslip was then sealed on all sides with paraffin and the preparation examined with the dark field microscope. An advantage of this method was that negative controls could be observed in the same preparation, since the reaction occurred only where the 2 reactants met, thus leaving the homogeneously dispersed globules of oil on one side and the serum on the other side.

A sample result is shown in Table I and Fig. 1.

The results obtained show that it is possible to adsorb the neutral extractable protein and the acid-soluble type-specific protein of

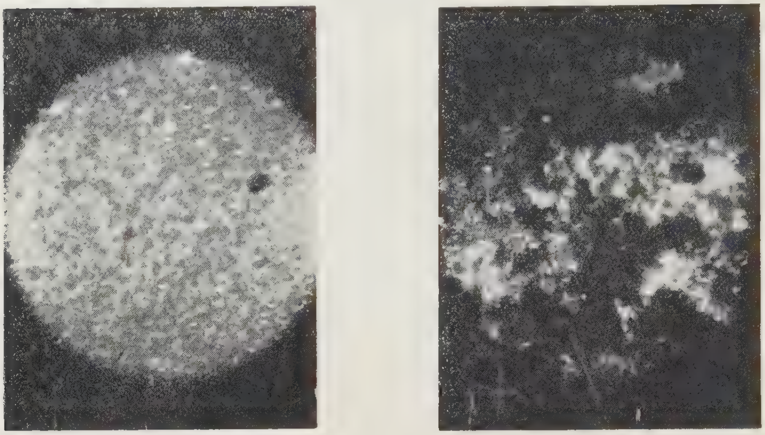
TABLE I.
Slide Agglutination.

Antigen: Olive oil emulsion of Type 1 and Type 6 acid-soluble protein (Lancefield's "M," type-specific).
Antisera: Rabbit sera against Type 1 and Type 6 whole streptococci.

		Dilutions of antiserum						
Antiserum	Antigen	1/2	1/4	1/8	1/32	1/64	1/128	1/256
Type 6	Type 6 "M"—oil	4	4	4	3	1	1	0
" 6	" 1 " "	1	0	0	0	0	0	0
" 1	" 6 " "	0	0	0	0	0	0	0
" 1	" 1 " "	2	4	3	2	1	0	0
Normal rabbit serum	" 6 " "	0	0	0	—	—	—	—
Normal rabbit serum	" 1 " "	0	0	0	—	—	—	—

Examples of

A negative reaction A positive reaction



Protein adsorbed on oil emulsion and normal rabbit's serum

Protein adsorbed on oil emulsion and immune rabbit's serum

FIG. 1.

Streptococcus pyogenes upon droplets of olive oil in emulsion, and that such droplets react with appropriate antiserum in much the same manner as an agglutinogen.

11176

Further Studies of the Agent in Intestines of Normal Mice Which Induces Encephalomyelitis.

PETER K. OLITSKY.*

From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

It has been shown[1] that normal mice, as well as those affected by spontaneous encephalomyelitis (Theiler's disease—now commonly called "poliomyelitis of mice"[2, 3]), harbor in their intestinal contents an active agent which, after cerebral transfer to normal mice, induces the characteristic signs of spontaneous encephalomyelitis. It was demonstrated[1] that cross-active immunity exists between the mouse-passaged experimental disease, originally derived from spontaneous encephalomyelitis, and the malady brought about by the active agent found in the intestines of normal mice.[1, 4] In view of this and further evidence already brought to light, it would seem that the latter is a strain of Theiler's virus, or is closely related thereto. It should be added that since that time no definite instance of the spontaneous, paralytic disease has been noted, although more than 4000 normal mice or those injected intracerebrally with a wide variety of different infective agents have been under observation during this period.

---

* Thanks are due to Dr. M. Theiler of the International Health Division of the Rockefeller Foundation for his cordial cooperation, and to Mr. P. Haselbauer for his invaluable technical assistance.

[1] Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 434.

[2] Theiler, M., *Science*, 1934, **80**, 122; *J. Exp. Med.*, 1937, **65**, 705.

[3] Iguchi, M., *Kitasato Arch. Exp. Med.*, 1939, **16**, 56.

In the first paper[1] reference was made to certain properties and epidemiological factors of the transmissible agent occurring normally in the intestinal tract of mice. The present communication will summarize the results of additional findings on the characteristics of this intestinal incitant of encephalomyelitis.

Methods. The methods employed were the same as those already reported.[1] In all experiments 8 to 10% of intestinal contents ground in broth and filtered through "V" Berkefeld candles was prepared and of the filtrate 0.03 cc was injected intracerebrally† into each of a selected number of 30-day-old mice.[1] The encephalomyelitis which developed was determined as specific by transfer of suspensions of bacteria-free cerebral tissues to fresh mice, or the presence of characteristic pathological changes resembling those of Theiler's disease, or by both methods.

Throughout the past year and at different periods within that time, we have succeeded in finding evidence of the presence of the active material in the intestinal tract of every normal mouse or groups of mice (in experiments with pooled material) examined for this purpose providing the animals were young adults. The results summarized in Table I serve to demonstrate this finding. Thus, in 18 different experiments, the intestinal contents of 60 mice, filtrates of which[1] were injected intracerebrally into a total of 130 fresh mice, were found to be characteristically active in every trial. It would, therefore, appear that normal mice of a certain age (Rockefeller Institute strain of albinos were employed throughout this study) harbor this agent invariably in their intestinal contents.

In 2 recent experiments, all of 8 control mice used in each test developed the experimental affection after receiving, intracerebrally,

---

[4] Theiler, M., and Gard, S., personal communication.

† Full ether-anesthesia was employed in operations on mice.

TABLE I.

Age at Which Virus Can Be Detected in Intestinal Contents of Normal Mice.

Age	Experiment	No. of mice used as source	Contents injected cerebrally		Remarks
			No. of mice	No. showing encephalomyelitis	
Fetus	A	6	8	0	Mothers of A and B groups pregnant about 16 days when fetuses removed. Virus in mothers' intestinal contents
"	B	8	8	0	
4 hr		4	8	0	
24 "	A	5	8	0	Mothers of A and B groups, virus in intestinal contents
" "	B	5	8	0	
2 days		3	8	0	
5 "	A	5	8	0	Mothers of A and B groups, no virus in pooled intestinal contents; their age, 6 months or older (see below for record of older mice)
" "	B	5	8	0	
" "	C	4	10	0	
12 "	A	5	8	0	Mothers of A and B groups, virus in intestinal contents
" "	B	5	8	0	
20 "	A	4	8	0	
" "	B	4	8	7	
" "	C	4	10	0	
" "	D	4	16	1	
25 "	A	4	8	7	
" "	B	4	8	1	
30 "		60	130	123	Summary of 18 different experiments (usually pooled 3 mice for each) and all positive
6 months or older	A	3	8	2	All females injected
	B	3	8	2	" males "
	C	2	4	2	
	D	2	8	3	
	E	2	7	0	
	F	2	8	5	

filtered intestinal contents which had been derived from normal mice. On the other hand, only 3 of 8 mice, in both tests, given suspensions of the corresponding intestinal walls (7-8% concentration) exhibited encephalomyelitis. The walls in the first trial were washed in tap water for 2 minutes and in the second, for 1 hour in 4 changes of saline solution (80 cc for each washing). We have thus confirmed an earlier observation on the activity of intestinal walls.[4] Further work is in progress to define more exactly whether suspensions of walls are disease-producing because of the adherence to the sides of the contents of the lumen, or because the active agent is located within the walls themselves.

Age in Relation to Presence of the Intestinal Agent. It will be seen from Table I that the active agent is not demonstrable in the intestines of fetal mice, nor at later periods of life, until some time after the 12th day of age—and the negative results occur even when mothers harbor it in their own intestinal contents. Thereafter, on the 20th day of age, it is detectable irregularly, until the 30th day, when it is invariably found. In old mice, on the other hand, it is still recoverable but again irregularly.

Normal mice, therefore, apparently harbor the active agent in their intestinal contents beginning at some short period after weaning. Experiments thus far made indicate that mothers' milk contains no substance that neutralizes the effects of the agent (equal parts of fresh milk, expressed on the 5th day of nursing, plus a suspension of 5% active brain, induced disease in all of 8 mice injected intracerebrally with the mixture kept at room temperature for 30 minutes—a similar response having been obtained in control mice in which broth replaced the milk). In addition, the milk by itself (20% in broth) failed to reveal the presence of any specific activity. It was shown previously[1] that feeding active brain-material failed to induce disease and in a recent experiment, the same food serving as rations for the mice after weaning, when mixed and suspended in tap water and filtered, the filtrate then being inoculated intracerebrally into 30-day-old mice, likewise did not bring about the experimental malady. Further work is being considered for elucidation of the problem which is: How does the active agent get into the intestines, and why does it become detectable shortly after the suckling period is over?

It is of interest that older mice, *i. e.,* those 6 months of age or older, apparently harbor less of the intestinal agent than do young adults. This circumstance may be correlated with the finding of Theiler[2] that old mice are resistant to intracerebral inoculation of his virus. In our hands, 1 :10 dilutions of brains of mice having encephalomyelitis, induced by cerebral injection of the intestinal agent, brought down with disease 10 of 10 30-day-old mice; 7 of 9 4-months-old, and 6 of 10 6-months-old ones. Whether this is due to prolonged carriage of the agent in the intestines, giving rise to an immune response, or to some other factor, is yet to be determined.

It was also shown that pregnant mice, artificially infected in the same way as the old animals just mentioned, developed encephalomyelitis 1, 5, and 12 days after the births of litters. The litter-siblings (total 15 young) were tested for the presence of the intestinal agent at birth and at 2, 5, and 20 days of age. The intestinal contents of 3 or 4 mice were pooled in each age group. Only the 20-day-old animals were positive. Since in this series no such agent was found until the young reached 20 days of age (Table I), it would appear that they were not affected by the presence of the active agent in their mothers during pregnancy, either as a result of artificial, intracerebral inoculation, or, as already mentioned, of their harboring it naturally in their intestines.

Absence of Agent in Brains of Normal Mice. The brains of 32 normal mice, the intestinal contents of which yielded the active agent, failed to exhibit any specific effect on intracerebral passage to 52 normal mice. In other words, while the encephalomyelitis-producing agent can be found invariably in the intestinal tract of normal mice of a certain age, their brains remain free from it.

Conclusions. Certain additional characteristics have been described of the active agent which is present in the intestines of normal mice and which can induce encephalomyelitis indistinguishable from that of the spontaneous Theiler's disease. Among other findings, it was ascertained that, irrespective of whether mothers harbor virus (by means of artificial, cerebral inoculation or naturally in their intestinal contents), the incitant is not detectable in the intestines of their young from the fetal stage to 12 days of age, but it is demonstrable at the age of 20 days or older. Moreover, at 30 days of age, practically every mouse that has come under observation has yielded specifically active intestinal contents. By some mechanism not as yet understood, old mice harbor less of the agent than do young adults. Finally, in the latter age-group, while the mice carry regularly the active substance in their intestines, it is not at all recoverable from their brains.

11177 P

Sleep Induced by Subthalamic Lesions with the Hypothalamus Intact.

H. R. MILLER AND E. A. SPIEGEL.

From the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa., and Montefiore Hospital, New York City.

The sleep-producing effect of hypothalamic lesions has been convincingly demonstrated (Ranson[1]). There are, however, still many unsolved problems which bear upon the central mechanism of the sleep-waking process. Supporting the view, notably that of Kleitman,[2] that sleep primarily depends upon a reduction of afferent impulses, Bremer[3] demonstrated that a transection of the brain stem behind the 3rd nuclei produces in the electrocorticogram and in the behavior of the eyeballs signs characteristic of sleep. Extensive bilateral thalamic lesions which destroyed the chief endings of the centripetal pathways from the spinal cord and rhombencephalon (Ranson) failed, however, to abolish the normal waking state. This indicates that centripetal systems outside the dorsal thalamus play an important part in maintaining the waking state. Thalamic lesions extending more ventrally than those of Ranson, reaching beyond the dorsal thalamus, but remaining outside the hypothalamus, were produced by Spiegel and Inaba,[4] who observed prolonged sleep following such lesions. The region ventrally adjacent to the thalamus, the so-called subthalamus, contains important fiber connections with the hypothalamus (Woollard,[5] and Papez[6]). It therefore seemed pertinent to investigate whether lesions of the subthalamus, sparing the hypothalamus, would interfere with the normal sleep-waking rhythm.

---

[1] Ranson, S. W., *Arch. Neur. and Psych.*, 1936, **35**, 1175; 1939, **41**, 1.

By means of the Horsley-Clarke stereotaxic apparatus the thalamus was pierced on both sides and bilateral circumscribed lesions were placed in the subthalamus. In control experiments lesions were confined to the thalamus or the hypothalamus. Experiments in all were made on 25 cats. Regardless of the anesthetic used (ether or nembutal), the cats with subthalamic lesions exhibited sleep (average duration 4 days). There was usually first deep sleep from which the animals could not be awakened and then somnolence associated with catatonia. In some animals the period of sleep was followed for about an equal length of time by a catatonic state without somnolence, the whole disturbance lasting up to 7-9 days. In other animals catatonia was the only disturbance. The histological examination of the sleep-producing punctures revealed that they passed through the posterior part of the thalamus, at the level of the ganglion habenulae and of the corpora mammillaria and immediately in front of these ganglia, traversed the medial part of the subthalamus, remaining lateral to the Vicq d'Azyr bundle, and ending dorsal to the inner end of the cerebral peduncle. The hypothalamus was histologically intact. Lesions limited to the thalamus proper failed to disturb the waking state. This was true also for punctures of the subthalamus at more rostral levels.

---

[2] Kleitman, N., *Physiol. Rev.*, 1929, **9**, 624.

[3] Bremer, F., *C. R. Soc. de biol.*, 1935, **118**, 1235; *Bull. Acad. de Med. de Belgique*, 1937, p. 68.

[4] Spiegel, E. A., and Inaba, C., *Z. f. d. ges. exp. Med.*, 1927, **55**, 164.

[5] Woollard, H. H., *Proc. Roy. Soc. Med.*, London, 1932, p. 1579.

[6] Papez, J. W., *Arch. Neurol. and Psych.*, 1937, **38**, 725.

Interference with the blood supply of the hypothalamus can play at most only a minor rôle since punctures with a dorsal entry, lateral to the hypothalamus and terminating at a considerable distance above the base of the brain, would hardly be expected to pierce blood vessels which go to the hypothalamus from the circle of Willis. Shock upon the hypothalamus may be partly responsible for the experimentally induced sleep, but it seems not sufficient cause to explain a disturbance lasting one week or longer (sleep plus catatonia in this series up to 9 days, sleep of 3 weeks' duration in Spiegel and Inaba's experiments on dogs). Besides it should be borne in mind that destruction of the hypothalamus itself also produces in cats disturbances of the waking state which are transitory only. The fact that unilateral hypothalamic punctures and even bilateral hypothalamic lesions when not extensive enough, fail to produce sleep, also speaks against the assumption that the symptoms observed after the subthalamic lesions are merely the consequence of shock upon the hypothalamus. It must, therefore, be concluded that damage of pathways passing through or synapsing in the subthalamus contributes to the genesis of the symptoms induced by the lesions. Ascending tracts conducting impulses from sensory spinal and cranial nerves as well as descending systems, particularly pallidofugal systems, must be considered. In this connection it is relevant to remark that sleep is produced by section of the midbrain and not by section behind the rhombencephalon (Bremer). In the former procedure ascending tracts from the cord and the rhombencephalon are severed as well as descending tracts from the higher centers. In the latter instance only the pathways ascending from and descending to the cord are interrupted. Since the majority of the descending hypothalamic tracts carries impulses to the vegetative centers in the cord and sleep does not follow a disruption of all descending systems entering the cord but is produced by a section interrupting the centripetal systems from the cord and rhombencephalon, it would seem that in the production of sleep by subthalamic injuries damage of ascending tracts plays an important part. The possibility, however, that lesion of the pallidofugal fibers may contribute to the production of the symptoms observed, particularly as regards the catatonic symptoms, cannot be denied.

11178 P

Allergic Reactions to an Antigen from the Chigger (*Trombiculum irritans*).

Arch Sherrod Morrow. (Introduced by F. H. Swett.)
From the Department of Anatomy, Duke University School of Medicine, Durham, North Carolina.

As it is common knowledge that through the summer months certain individuals are annoyed far more than others by the bite of the chigger, it was thought that the irritation might be an allergic response. With this in mind the following preliminary experiments were done.

Methods. Trombicula larvae which had not had access to man were collected, brought into the laboratory while still alive and ground in 0.2 cc of distilled water. The material was then diluted with distilled water so that each cc represented antigen from 100 chiggers. After allowing this solution to stand about 30 minutes, it was centrifuged at high speed for 20 minutes. The clear supernatant fluid was pipetted off and sterilized by autoclaving.

A second antigen was prepared and sterilized without the use of heat by passing the infusion of ground chiggers through a Seitz filter.

Results. The first antigen when injected intradermally gave a sharp stinging sensation as it went into the skin. There was produced an immediate wheal with surrounding area of erythema. The maximum wheal was developed in from 20 to 30 minutes, subsiding rather rapidly and disappearing within 2 to 3 hours. The size of the wheal varied with different individuals. One-tenth of a cubic centimeter in the skin of one subject gave a wheal (with pseudopods) 155 sq mm in area, and an area of erythema measuring 1658 sq mm, while a control of distilled water gave a wheal (with no pseudopods) measuring 38 sq mm and an erythematous area of 84 sq mm.

The injection of the second or filtered antigen, in addition to giving the sharp stinging sensation resulted in a pronounced itching not felt with the autoclaved material. The wheal produced by 0.1 cc of this antigen in the skin of the same subject mentioned above was larger, measuring 212 sq mm and the erythematous area measured 1839 sq mm.

A passive transfer experiment was done, in which 0.1 cc of serum from each of 3 donors (respectively very sensitive, moderately sensitive and relatively insensitive to chigger bites) was injected intradermally at 9 areas on the back of an individual not sensitive to bites

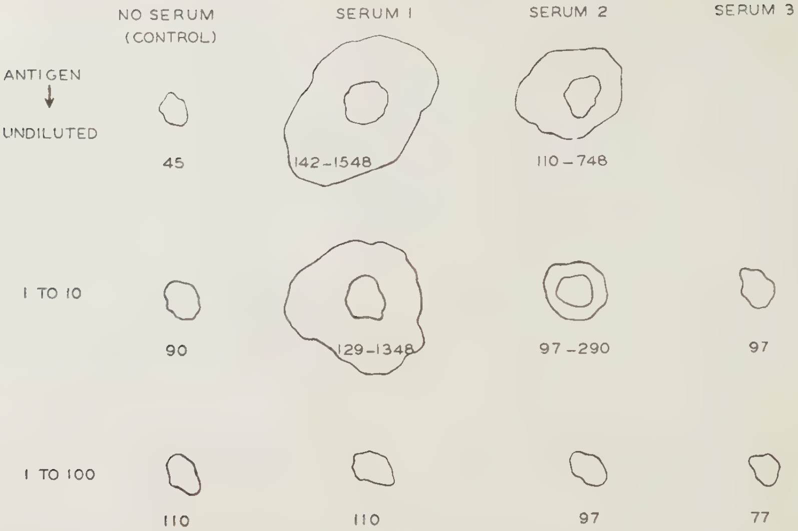


Fig. 1.

Reactions obtained with passive transfer. Measurements given in mm^2—first number is area of wheal, the second is area of erythema. × approximately ½.

of the larvae and 24 hours later the antigen was injected into the same areas. Full strength antigen and dilutions of 1 to 10 and 1 to 100 were used. Controls were run by injecting the antigen and the dilutions into the normal skin of the recipient. As is shown in Fig. 1, the response with the wheal and erythema varied with the different sera. There was a definite correlation between the response and the apparent sensitivity of the donor. Individual injections of the antigen into the skin of these same donors gave a corresponding gradation in size of wheal and erythema produced.

Discussion. As there is a definite difference in reaction to the autoclaved and the filtered antigen, it is reasonable to suspect that a part of the secretion of the chigger injected at the time of the bite is destroyed by heating.

It seems from the difference in the reactivity of the sera in the passive transfer that there is a marked variation in sensitivity in different individuals. Whether this response can be directly correlated with the reactivity to antigen injected directly in the individual's skin, and whether either or both can be correlated with the degree of irritation caused by the chigger bite must await further experimentation. The present study suggests that the extreme irritation produced by the bite of the chigger is due to previous sensitization of man to some substance introduced into the skin. If this can be proven, it is reasonable to look forward to the possible reduction of the irritation by desensitization with a suitable antigen.

Summary. An antigen has been prepared by grinding chiggers and extracting with distilled water. Certain individuals gave a positive skin reaction to this antigen. Heat seems to destroy a portion of the antigen so that it becomes less irritating and induces less reaction. A passive transfer was done with positive results.

11179 P

Pathology of Irradiation Sickness: A New Method for Inducing Shock.

VIRGIL H. MOON, KARL KORNBLUM AND DAVID R. MORGAN.
From Jefferson Medical College and Hospital, Philadelphia.

It is well known that exposure of the abdominal region to massive Roentgen therapy produces severe illness. Nausea, vomiting, oliguria, bloody diarrhea, rapid feeble pulse, low blood pressure and profound prostration follow. The metabolism is low, the blood sugar and N.P.N. are increased, the blood chlorides and the alkaline reserve decline. These clinical features are identical in character with those of shock. Illness does not develop immediately, but after an interval of 2 or 3 days. Death occurs about 4 days after exposure.

Irradiation sickness was produced in dogs in order to make observations on hemoconcentration and on the visceral changes. From 1400 to 2800 Roentgen units were given in divided doses over different parts of the abdomen. Severe illness developed after an interval of 60 to 72 hours and hemoconcentration, ranging from 15 to 50%, appeared. The urination was decreased and traces of blood were seen in the urine, feces and vomitus. This blood apparently came from capillary hemorrhages in the mucosae. It was not of sufficient amount to affect significantly the total blood volume. The illness progressed rapidly, and death by circulatory failure occurred within 24 hours.

The gross and microscopic findings were those characteristic of shock.[1, 2] These included capillo-venous congestion of the viscera, petechial hemorrhages in mucous and serous surfaces, edema of

---

[1] Moon, V. H., *Shock and Related Capillary Phenomena,* Oxford University Press, New York, 1938.

[2] Moon, V. H., *Arch. Path.,* 1937, **24**, 642, 794.

lungs and of mucosae, and parenchymatous degeneration of liver and kidneys. The results were concordant in 12 animals so treated.

In one series, the animals were treated as described, but were killed at intervals of 2, 2½, and 3 days after exposure. This was done in order to make examinations of the intestinal mucosae at earlier stages after the injury. Sections of bowel and of other tissues were placed in fixative within 5 minutes of the death of the dogs.

Microscopic study of such sections confirmed the findings of Whipple[3, 4] and others who have made similar studies. The evidences of damage were most marked in the mucosa of the small bowel. The epithelium lining the crypts and covering the villi, showed all stages of disintegration and destruction. Some crypts contained nuclear and cellular debris, others were empty. Some of the villi retained their epithelial covering while that in the crypts showed necrosis and disintegration. Other villi were completely denuded of their epithelial covering.

Our results and interpretations confirm those of Whipple and his associates: Exposure of the abdomen to prolonged deep X-ray treatment causes delayed necrosis of the intestinal mucosa. The development of irradiation sickness apparently is due to the absorption of products of disintegrating tissues.

These experiments have a significant bearing on the mechanism of shock. Some writers assign this to local hemorrhage and loss of fluid, or to sympatho-adrenal hyperactivity, and disregard absorption of products from damaged tissues as a factor. Injury to the intestinal mucosa by irradiation eliminates pain, emotional reactions, sympatho-adrenal hyperactivity, hemorrhage and anesthesia as factors. In the experiments described it appears that the disturbance of circulation resulted solely from absorption, independent of other factors.

Summary. Exposure of the abdomen to deep Roentgen irradiation causes physiologic disturbances like those of shock arising from other causes. This is accompanied by progressive hemoconcentration. The visceral findings post mortem are those characteristic of shock regardless of its origin. Deep Roentgen irradiation causes delayed necrosis of the intestinal mucosa. The resulting signs of illness are likewise delayed in onset.

Irradiation provides a new method for inducing shock by injury to living tissues. This method eliminates the confusing effects of other factors including pain, emotional reactions, sympatho-adrenal hyperactivity, hemorrhage and anesthesia.

---

[3] Hall, C. C., and Whipple, G. H., *Am. J. Med. Sc.*, 1919, **157**, 453.

[4] Warren, S. L., and Whipple, G. H., *J. Exp. Med.*, 1923, **38**, 713.

11180 P

Influence of Estrogen on Insulin Requirement of the Diabetic.

Anna R. Spiegelman. (Introduced by H. O. Mosenthal.)
From the Sea View Hospital, Staten Island, N. Y.

This study consists of a series of 9 diabetic women taken from the Metabolic Service of Sea View Hospital. The patients were chosen without regard for an indication for estrogen therapy. They were followed for a period of one year: 4 months before the beginning of estrogen, 5 months during its administration, and 3 months after it had been discontinued. Estrogen was administered intramuscularly in doses of 10,000 International Units twice a week for a period of 4 months.

During the 4 months before estrogen therapy a definite effort was made to maintain the individual insulin dosage at the lowest level compatible with proper control of the diabetes. A patient was considered well controlled when in the absence of acetonuria there was less than 1% of sugar in each of the partition specimens of urine and the total amount of sugar spilled in a period of 24 hours was less than 20 g. To meet these requirements it was necessary to increase our daily insulin dose steadily from 63 units at the beginning of this period of observation to 84 units at the end of this 4-month period.

Two weeks after estrogen was instituted the average daily insulin requirement started to show a significant decline so that during the 5 months in which our patients were receiving estrogen there was a drop in the daily average insulin requirement from 84 to 54 units, representing a saving of 35%.

At the end of this 5-month observation period estrogen was discontinued. The patients were then followed 3 months longer. There was a gradual increase from an average daily requirement of 54 units at the beginning of this time to 63 units at the end. This final figure is still 25% lower than the "pre-estrogen" level of 84 units daily.

The type of insulin used did not influence the results obtained during the administration of estrogen for the percentage of insulin saved in the patients receiving regular insulin and in those on protamine zinc insulin was identical. The patients on regular insulin showed a decrease in the daily average insulin dose of 47%, a drop from 117 to 60 units daily. The requirements for those on protamine zinc insulin showed a decrease from 64 to 34 units daily, again a saving of 47%.

The age of the patient, however, did influence the results obtained with the administration of estrogen. In the premenopausal patients there was a daily insulin saving of 63% represented by a decrease in the daily average insulin requirement from 48 units to 18 units. In the postmenopausal patients, on the other hand, the "pre-estrogen" requirement of 108 units daily fell only 41% to 63 units daily during the administration of estrogen.

11181

Diurnal Variations in the Acid-Base Balance.

A. BAIRD HASTINGS AND CHARLES W. EISELE.

From the Department of Biological Chemistry, Harvard Medical School, Boston, and the Department of Medicine, University of Chicago, Chicago.

Interpretation of abnormal variation in the acid-base balance of the blood presupposes a definition of what constitutes normal variations. There has been accumulated in the past a large amount of data on the pH, the serum bicarbonate concentration and the CO_2 tension of the blood of normal individuals. It is generally regarded that if, in arterial blood, the pH is between 7.35 and 7.45, the bicarbonate between 24.5 and 27.5 mM per liter, and the CO_2 tension between 40 and 45 mm, a normal acid-base balance of the blood is indicated.

Shock and Hastings,[1] in a series of 131 determinations on 57 normal individuals, found that the variation from individual to individual was no greater than the variation found in a single individual on repeated observations. Whether or not the acid-base balance of the blood of a single individual varies in any consistent manner during the course of a day is still an open question.

Cullen and Earle[2] made 5 or 6 determinations of the acid-base balance of the blood on 10 individuals between 9 in the morning and 9 at night. They came to the conclusion at that time that there tended to be an alkaline shift during the day, although this was not an invariable finding. Shock and Hastings made hourly observations on each of 10 subjects for a total of 22 experiments between the hours of 9 and 6. These individuals were permitted to go about their usual activities. Some shifts in CO_2 tension, in pH and bicarbonate were observed, but the changes were not consistently in any one direction. In view of those results and the great sensitivity of the acid-base balance of the blood to slight changes in respiratory activity, it was concluded that the variations encountered in single individuals during the course of a day of normal activity was largely a matter of chance.

---

[1] Shock, N. W., and Hastings, A. B., *J. Biol. Chem.*, 1934, **104**, 585.

[2] Cullen, G. E., and Earle, I. P., *J. Biol. Chem.*, 1929, **83**, 545.

However, whether or not variations of a consistent nature occur when a normal subject is at bed rest during a complete 24-hour period has not previously been studied. The present paper has, for its purpose, the presentation of the acid-base changes in the blood under such conditions.

Experimental. The subject was a normal individual convalescing from a minor surgical operation. Two 24-hour experiments were completed—the first, 6 days after the operation; the second, a week later. Samples of cutaneous blood, which closely approximates arterial blood in composition, obtained by the technic described by Shock and Hastings,[3] were collected at approximately 2-hour intervals throughout the 24-hour period. The 2 a. m. sample was omitted in both experiments, the 6. a. m. sample in the first experiment.

The blood was analyzed in duplicate for the percentage cells, pH_s, and total CO_2, using the micro acid-base method described by Shock and Hastings. The duplicate CO_2 analyses agreed within 0.6 mM/liter, the pH determinations within .01. From these data, the bicarbonate concentration of the serum $(HCO_3)_s$ and the CO_2 tension of the blood (pCO_2) were calculated. By estimating what the serum bicarbonate concentrations would have been at pH 7.40 [designated as $(HCO_3)'_s$ and calculated from the equation, $(HCO_3)'_s = (HCO_3)_s + 30\ (pH—7.40)$] it was possible to determine whether any trends toward net base excess or net acid excess occurred during the 24-hour cycle. These data are given in Table I.

Comment. In experiment 1, the pH varied from 7.31 to 7.43; the $(HCO_3)_s$ from 24.1 to 28.0 mM per liter; the $(HCO_3)'_s$ from 24.2 to 28.9 mM per liter; and the pCO_2 from 38.3 to 55.2 mm Hg. In experiment 2, the pH variation was from 7.36 to 7.42; the $(HCO_3)_s$ from 26.4 to 30.3; the $(HCO_3)'_s$ from 25.2 to 29.7; the pCO_2 from 45.2 to 53.8. Such variations as these are as great as one would encounter in a series of single determinations of a large number of normal individuals. It was found that the variations were not random, however, but represented trends of a definite nature, in excess of the errors of observation.

These trends have been shown in Fig. 1 by plotting against time,

---

[3] Shock, N. W., and Hastings, A. B., *J. Biol. Chem.*, 1934, **104**, 565.

TABLE I.

Determination of Percentage Red Cells, V_c; pH_s; CO_2 Content of Blood $(CO_2)_b$, During 24-hour Period. Subject at Rest in Bed. Food at 8:30 a.m., 12:30 p.m., 5:30 p.m.; Sleep, 10:30 p.m. to 8:00 a.m.

	Experiment 1						Experiment 2					
	Determined			Calculated			Determined			Calculated		
Time	V_c	pH_s	$(CO_2)_b$ mM/L	$(HCO_3)_s$ mM/L	$(HCO_3)'_s$ mM/L	pCO_2 mm Hg	V_c	pH_s	$(CO_2)_b$ mM/L	$(HCO_3)_s$ mM/L	$(HCO_3)'_s$ mM/L	pCO_2 mm Hg
a.m.												
8	.44	7.42	20.7	24.1	24.7	38.3	.48	7.38	23.4	27.6	27.0	48.1
10	.44	7.41	22.9	26.7	27.0	43.4	.47	7.40	24.0	28.4	28.4	47.3
12	.49	7.43	22.6	27.2	28.1	42.3	.47	7.41	24.2	28.6	28.9	46.6
p.m.												
2	.43	7.43	24.0	28.0	28.9	43.5	.47	7.40	23.4	27.6	27.6	46.0
4	.44	7.41	23.9	27.8	28.1	45.3	.44	7.42	24.4	28.4	29.0	45.2
6	.44	7.41	21.7	25.2	25.5	41.1	.45	7.36	22.8	26.4	25.2	48.1
8	.40	7.42	22.9	26.2	26.8	41.7	.46	7.39	22.8	26.7	26.4	45.5
10	.43	7.40	21.8	25.1	25.1	41.8	.46	7.38	24.3	28.4	27.8	49.5
12	.44	7.40	22.1	25.7	25.7	42.7	.45	7.36	25.5	29.5	28.3	53.8
a.m.												
4	.44	7.31	23.8	26.9	24.2	55.2	.46	7.37	24.8	28.9	28.0	51.8
6	—	—	—	—	—	—	.46	7.38	24.4	28.5	27.9	49.8
8	.45	7.35	22.7	26.0	24.5	48.5	.46	7.38	25.9	30.3	29.7	52.8
Mean		7.40	22.6	26.3	26.2	44.0		7.39	24.2	28.3	27.9	48.7
Min.		7.31	20.7	24.1	24.2	38.3		7.36	22.8	26.4	25.2	45.2
Max.		7.43	23.9	28.0	28.9	55.2		7.42	25.9	30.3	29.7	53.8

the deviation from the mean value for the 24-hour period of the CO$_2$ tension (pCO$_2$), and of the bicarbonate concentration at pH 7.40, (HCO$_3$)$'_s$. The experimental data are shown by points—the mean values for the two experiments by solid lines. Considering the small

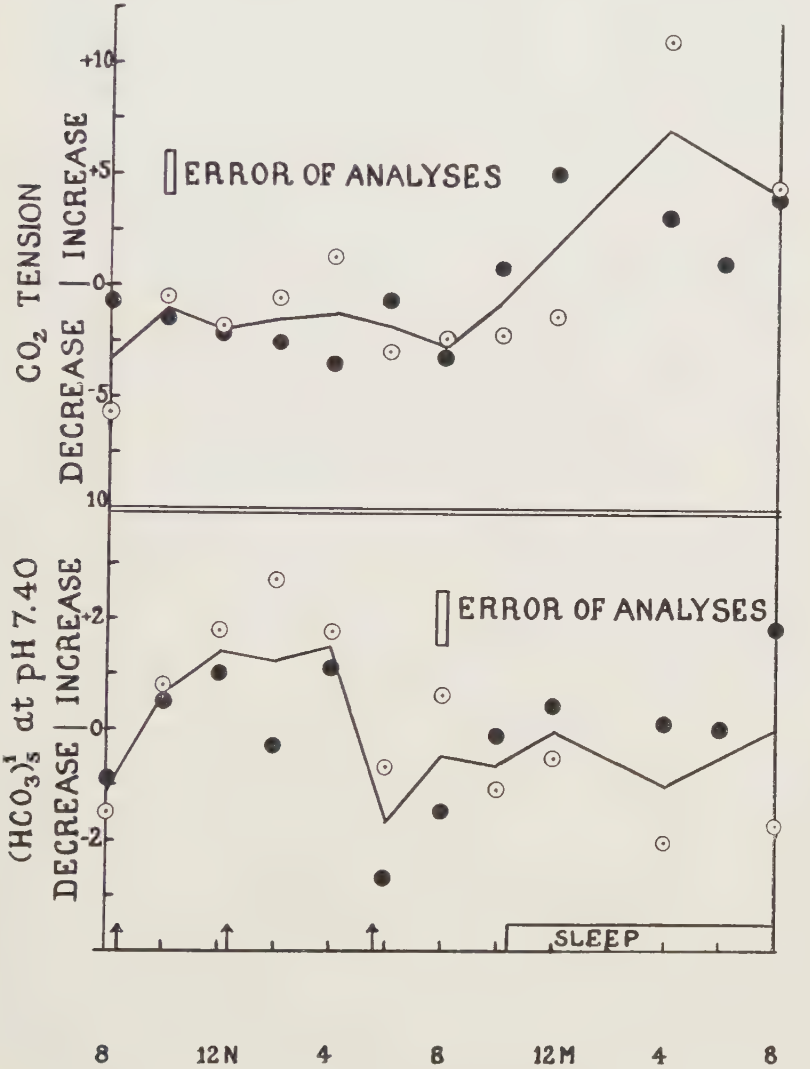


FIG. 1.

The upper chart shows the changes in pCO$_2$ with time; the lower chart, the changes in (HCO$_3$)$'_s$ with time. The data of experiment (1) are designated as ⊙; of experiment (2), as ●. The solid lines indicate the mean data of both experiments. The extent of the maximum analytical errors on pCO$_2$ and (HCO$_3$)$'_s$ are indicated by the open rectangles. The arrows indicate the ingestion of food.

magnitude of the changes involved, the two experiments are in agreement.

The data showed certain trends in the blood during the day which seem quite definite: (1) The value of $(HCO_3)'_s$ rose about 2 millimols during the forenoon and descended to its original value in the afternoon. In one experiment, the $(HCO_3)'_s$ rose again during the night; in the other, it did not. In 5 out of 6 samples taken from 1.5 to 2 hours after eating, there was an increase in $(HCO_3)'_s$ amounting to approximately 1 mM. This parameter of the acid-base balance, $(HCO_3)'_s$, is determined by the net difference between the equivalent concentrations of basic ions and acid ions (other than HCO_3) at pH 7.40. Changes in $(HCO_3)'_s$ may result, therefore, from a departure in the balance between production or absorption, and destruction or excretion of the acidic and basic components of the blood. Since physical activity was absent as a disturbing factor in these observations, it is reasonable to assume that the trend toward alkali increase after meals was due to the combined effect of the process of digestion and the acid-base balance of the food ingested.

(2) The CO_2 tension showed little variation during the day until the subject went to sleep. There was then a rise amounting to approximately 7 mm of Hg over the average CO_2 tension when awake. Indeed, the analyses made in the first experiment on blood drawn at 4 a. m. showed a rise of 11 mm above the mean value for the day. This is in agreement with the observations of Endres,[4] who found that the CO_2 tension of alveolar air, and hence, of arterial blood, is increased in sleep.

Conclusion. The acid-base balance of the blood of a normal adult at rest has been found to vary (in a regular, not random manner) during a 24-hour period due to the following factors: (a) a rise in plasma HCO_3 associated with the ingestion of food; (b) an increase in the CO_2 tension of the blood associated with sleep.

---

[4] Endres, G., *Biochem. Z.*, 1923, **142**, 53.

11182 P

Effect of Nerve Section upon Development of Nutritional Muscular Dystrophy in Young Rats.

Alwin M. Pappenheimer and Marianne Goettsch. (With the assistance of Claudia Karsubova.)

From the Departments of Pathology and of Biological Chemistry, Columbia University, New York City.

It is now well established that the young of female rats maintained on a diet restricted in Vitamin E, develop, towards the end of lactation, severe degeneration of the skeletal muscles. The pathological changes have been described in detail by Olcott,[1] by Pappenheimer,[2] and by Telford, Emerson and Evans.[3] Barrie[4] and Olcott found no abnormalities of the nerves, spinal cord or brain, and the spinal cord changes described by Lipschütz[5] in young rats on E-deficient diet, have not been present in our material. We have noted that even the terminal neurites and motor end plates are well preserved in the midst of the degenerating muscle fibers.

Although the histological evidence thus favors a "primary myopathy" as against a neural origin of the muscle lesions, so little is known of the pathogenesis of this disease that it seemed of interest to ascertain whether the muscle degeneration would be affected by the removal of nervous impulses.

Muscular dystrophy was induced in young rats according to the technic described by Goettsch and Ritzmann.[6] Under ether anesthesia, the left sciatic nerve was sectioned through an incision on the posterior surface of the thigh, a segment several mm in length being excised. The operation was performed on various days during the lactation period—the earliest at 5 days, the latest at 18 days. The animals were killed soon after the development of symptoms, or when these were not manifest, upon the 24th day. It has been our experience that muscle lesions may be found in rats which have shown no clinical evidence of the disease. The operation was performed upon 42 rats, of which 29 developed the disease.

---

[1] Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

[2] Pappenheimer, A. M., *Am. J. Path.*, 1939, **15**, 179.

[3] Telford, I. R., Emerson, G. A., and Evans, H. M., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 291.

[4] Barrie, M. M. O., *Lancet*, 1937, **2**, 251.

[5] Lipschütz, M. D., *Rev. Neurol.*, 1936, **65**, 221.

[6] Goettsch, M., and Ritzmann, J., *J. Nutrition*, 1939, **17**, 371.

TABLE I.
Effect of Section of Left Sciatic Nerve upon Muscular Dystrophy of Young Rats on Vitamin E Deficient Diet.

Serial No.	Litter No.	Age at operation in days	Age at death in days	Lesions R. gastrocnemius	 L. gastrocnemius	 Other muscles
1	234 a	5	21	++++	—	++++
2	199 a	7	22	++++	—	++++
3	200 a	12	19	++++	—	++++
4	c	12	20	++++	—	++
5	189 a	15	16	±	—	—
6	b	15	18	+ (?)	+++ (?)	++++
7	196 a	15	20	+++	—	+++
8	b	15	20	+++	—	+++
9	c	15	24	++++	—	+++
10	e	15	24	±	—	—
11	g	15	22	+++	—	—
12	h	15	22	+++	—	—
13	i	15	23	+	—	—
14	218 a	15	24	±	—	+
15	b	15	23	+++	—	+++
16	c	15	24	+++	—	+++
17	d	15	23	+++	—	+++
18	240 a	16	21	++++	+	++++
19	b	16	21	++++	—	++
20	217 a	17	19	++++	—	+++
21	b	17	22	++++	—	+++
22	216 a	17	25	++++	—	++++
23	224 a	18	20	+++	+++	+++
24	b	18	20	+++	+++	+++
25	c	18	20	+++	+++	+++
26	d	18	25	+++	+++	+++
27	e	18	25	—	+++	+++
28	244 a	18	20	++++	++++	++++
29	b	18	25	++++	—	—

The right and left gastrocnemius muscles were fixed in Zenkers fluid and compared histologically.

The results of these experiments, though unexpected, were very definite and consistent (Table I). On the right side, the gastrocnemius with intact nerve supply showed the usual intense lesions—hyaline necrosis and segmentation of fibers, interstitial oedema, polymorphonuclear and histiocytic reaction, activation and proliferation of myoblasts (Figs. 2a and 3a).

In sharp contrast to these necrotizing and inflammatory lesions, the left gastrocnemius which had been deprived of its nerve supply was found to show only the familiar alterations consequent upon the nerve section—reduction in the caliber of the fibers, concentration, swelling and often central location of the nuclei (Fig. 2b). The degeneration or complete disappearance of terminal neurites and motor end plates was confirmed in Cajal preparations.

From Table I, it will be seen that when the operation is deferred

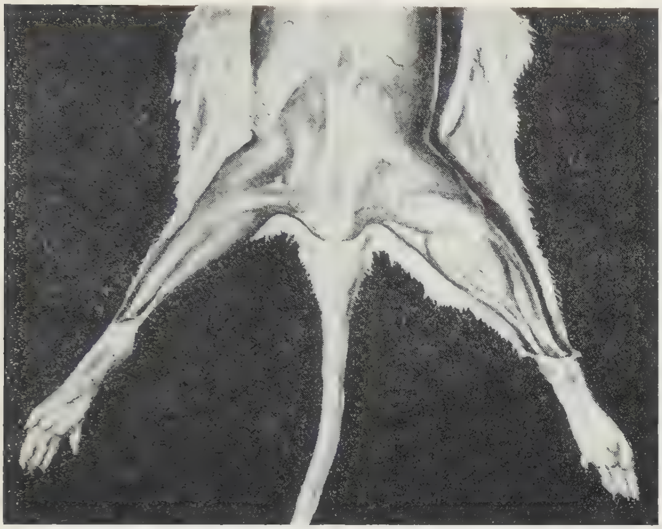


FIG. 1.

Rat 216a. Age 25 days. L. sciatic nerve sectioned on 17th day. R. gastrocnemius—swollen, streaky white, marked necrosis ++++. L. gastrocnemius—slight atrophy, otherwise normal in appearance.

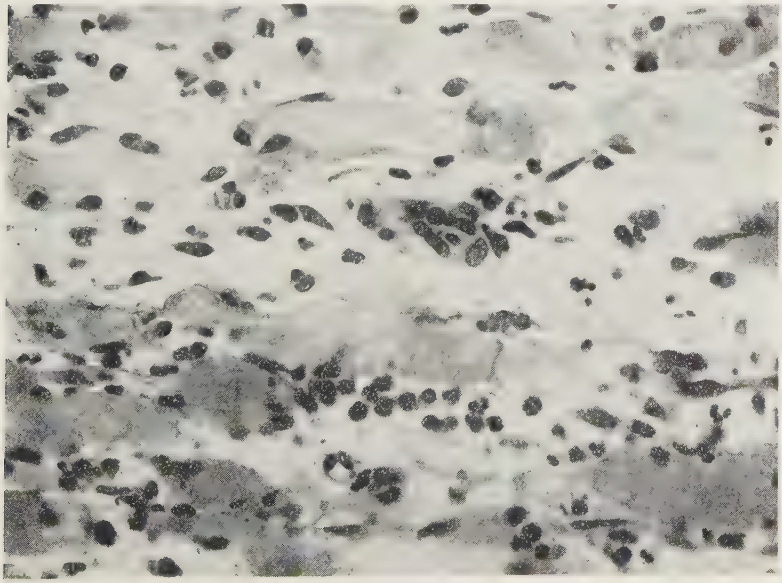


FIG. 2.

a. Rat 200c, age 20 days. Right gastrocnemius. Hyaline necrosis, segmentation, interstitial oedema, acute inflammatory reaction.

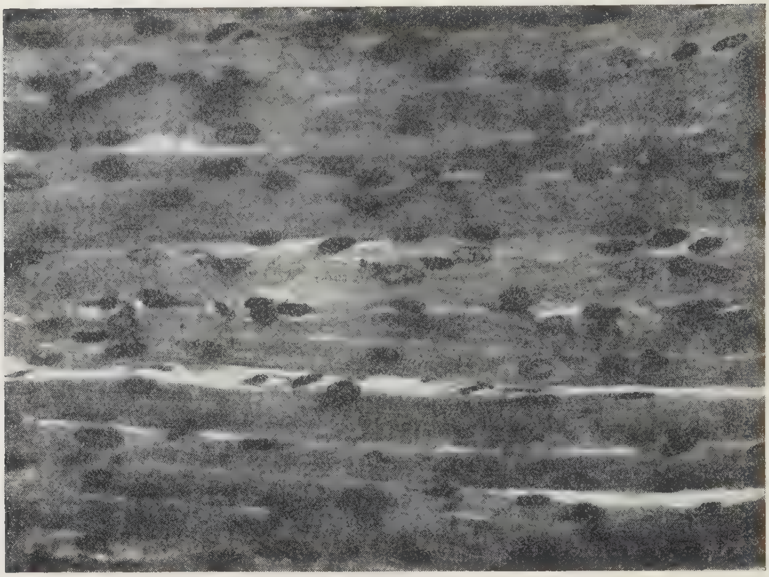


FIG. 2.

b. Rat 200c, age 20 days. Left gastrocnemius. Slight atrophy, otherwise normal fibers. Left sciatic nerve had been sectioned on 12th day.

until the 18th day, nerve section no longer confers protection. The failure to prevent the lesions by nerve section when the operation is carried out within 24 to 48 hours of the onset of the disease, may be explained by the persistence of excitability of the nerve during this period (Tower[7]).

One may safely conclude from these experiments that removal of nerve impulses protects the muscles of young rats from the usual effect of Vitamin E deficiency. The exact mechanism by which this protection is afforded—particularly the part played by the loss of sympathetic and motor impulses respectively—can be determined only by further experiments.

---

[7] Tower, S., *Phys. Reviews*, 1939, **19**, 1.

11183

Thiazol Derivatives of Sulfanilamide and Experimental Beta-Hemolytic Streptococcal and Pneumococcal Infections in Mice.

O. W. Barlow and E. Homburger.

*From the Research Laboratories of the Winthrop Chemical Company, Inc.,
Rensselaer, New York.*

In a previous paper[1] the specific chemotherapeutic action of 2-sulfanilamidothiazol (sulfathiazol) and 2-sulfanilamidomethylthiazol (sulfamethylthiazol) in experimental *Staphylococcus aureus* infections has been described. This communication presents a comparison of the efficiency of sulfathiazol, sulfamethylthiazol and 2-sulfanilamidophenylthiazol (sulfaphenylthiazol) with that of sulfanilamide

TABLE I.
Chemotherapy: Beta-hemolytic Streptococcus (Mice).

No. of mice	Preparation	Dosage* Single, mg/kg	Dosage* Total, g/kg	Survival in hrs Range	Survival in hrs Median	Survival 10 days % of series
60	None (controls)			10-25	14.25	0
40	Sulfanilamide	25	0.35	64-240+	152	30
40	,,	100	1.40		240+	100
40	,,	250	3.5		240+	100
40	Sulfapyridine	10	0.14	31-240+	64	30
50	,,	25	0.35	20-240+	240+	80
50	,,	100	1.40		240+	100
50	,,	250	3.5		240+	100
40	Sulfathiazol*	14.8	0.192	30-47	44	20
40	,,	37.0	0.481	54-114	240+	80
40	,,	148.0	1.92		240+	100
40	,,	370.0	4.81	120	240+	90
40	Sulfamethylthiazol	10	0.14	40-240	91	30
50	,,	25	0.35	68-240	240+	88
50	,,	100	1.40		240+	100
50	,,	250	3.5		240+	100
40	Sulfaphenylthiazol	10	0.14	69-240	87	40
50	,,	25	0.35	90-240	240+	90
50	,,	100	1.4		240+	100
50	,,	250	3.5		240+	100

* All of the dosage figures represent the actual amounts of each compound. The doses of sulfathiazol in Table I, and of sulfathiazol and sulfaphenylthiazol in Tables II and III, were calculated for comparative purposes on the basis of their sulfanilamide equivalents.

---

[1] Barlow, O. W., and Homburger, E., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 792.

and sulfapyridine in experimental beta-hemolytic streptococcus and pneumococcus Types I, II, and III infections.

Method. Male albino mice of uniform age from a standard strain weighing between 19 and 21 g were inoculated intraperitoneally with 0.3 cc of a saline suspension of an 18-hour culture of beta-hemolytic streptococcus (Lancefield Group A) C-203 of proven high mouse virulence representing from 0.75 to 1.0 million organisms. In a test of the virulence of this culture, 100 organisms proved fatal to all of a group of 20 mice. The dosage, therefore, represented from 7,500 to 10,000 lethal doses. Treatment was carried out 1½, 7, 12, 24, 32, and 48 hours after inoculation, and once daily thereafter until death occurred or up to and including the tenth day. The preparations were administered as a suspension in ¼ to ½ cc of milk (per single dose) by means of the stomach tube.

Results. The effects of sulfanilamide and various derivatives on beta-hemolytic streptococcus infections in mice are illustrated in Table I.

Tables II, III, and IV indicate the effectiveness of the thiazol compounds in the treatment of experimental pneumococcal infections of various types in albino mice.

In the tests summarized in these tables, all mice were inoculated with 0.3 cc of a saline suspension of 24-hour mouse virulent serum dextrose culture of pneumococci. Typed strains were obtained from

TABLE II.
Chemotherapy: Pneumococcus Type I (Mice).

No. of mice	Preparation	Dosage Single, mg/kg	Total, g/kg	Survival in hrs Range	Median	Survival 10 days % of series
40	None (controls)			19-75	30.1	0
50	Sulfanilamide	1000	14.0	42-190	84	0
40	Sulfapyridine	250	3.5	64-240+	82	20
40	”	500	7.0	86-240+	97	30
50	”	1000	14.0	89-240+	240+	50
30	Sulfathiazol	370	5.18	34-240+	47	10
40	”	740	10.36	48-240+	62	20
40	”	1480	20.72	27-240+	139	40
40	Sulfamethylthiazol	250	3.5	69-240+	84	20
40	”	500	7.0	82-240+	129	30
40	”	1000	14.0	90-240+	240+	52
40	Sulfaphenylthiazol	480	6.7	35-190	47	0
40	”	960	13.4	39-240+	67	10
50	”	1920	26.88	40-240+	69	20

TABLE III.
Chemotherapy: Pneumococcus Type II (Mice).

No. of mice	Preparation	Dosage		Survival in hrs		Survival 10 days % of series
		Single, mg/kg	Total, g/kg	Range	Median	
60	None (controls)			29-56	37	0
50	Sulfanilamide	250	3.5	44-111	52	0
50	,,	500	7.0	62-133	82	0
50	,,	1000	14.0	82-235	124	0
40	Sulfapyridine	250	3.5	50-220	78.5	0
40	,,	500	7.0	48-240+	87	12.5
40	,,	750	10.5	79-240+	109	22.5
50	,,	1000	14.0	75-240+	132	30.0
30	,,	1500	21.0	92-240+	168	23.3*
20	Sulfathiazol	370	5.18	52-240+	69	10
20	,,	740	10.36	78-240+	90	25
20	,,	1480	20.72	69-240+	136	30
40	Sulfamethylthiazol	250	3.5	65-186	70	0
40	,,	500	7.0	74-240+	97	15
40	,,	750	10.5	72-240+	104	20
40	,,	1000	14.0	75-240+	154	27.5
40	,,	1500	21.0	110-240+	182	30
30	Sulfaphenylthiazol	480	6.72	57-142	74	0
30	,,	960	13.44	60-240+	93	10
30	,,	1920	26.88	70-240+	205	26.6

* Animals showed evidence of toxic effects from sulfapyridine.

the Maryland State Health Department, Baltimore, Maryland. Treatment was carried out by stomach tube at the time intervals indicated for the antistreptococcus tests.

The antistreptococcal actions of the thiazol derivatives of sulfanilamide in experimental beta-hemolytic streptococcus infections in mice, as based on the percentage of survivals in the lower dosage group of animals, were 2 to 3 times as effective as sulfanilamide and equal to those of sulfapyridine.

The therapeutic efficiency of sulfathiazol, sulfamethylthiazol or sulfapyridine on oral administration by tube to mice infected experimentally with L 100 as well as L 70 suspensions of pneumococcus Types I, II, III was essentially the same as indicated by the incidence of survivals. All 3 compounds were definitely (2 to 7.5 times) superior to sulfanilamide in the treatment of experimental pneumococcic infections. Sulfaphenylthiazol was superior to sulfanilamide, but appeared to be somewhat inferior to sulfapyridine or to either the unsubstituted thiazol or the methyl substituted thiazol derivatives.

In order to determine the blood concentration range required for

TABLE IV.
Chemotherapy: Pneumococcus Type III (Mice).*

		Dosage		Survival in hrs		
No. of mice	Preparation	Single, mg/kg	Total, g/kg	Range	Median	Survival 10 days % of series
20	None (controls)			21-39	27	0
20	Sulfapyridine	250	1.75- 3.5	59-240+	89.5	5
20	,,	500	3.5 - 5.0	88-142	121	0
10	,,	750	5.25- 7.5	80-140	110	0
35	,,	1000	7.0 -14.0	82-240+	183	20
10	Sulfathiazol	250	1.25- 1.75	36-86	48	0
10	,,	1000	7.0 -14.0	79-240+	142	20
10	Sulfamethylthiazol	250	1.5 - 2.25	54-120	87.5	0
10	,,	750	5.25- 7.5	85-152	127	0
15	,,	1000	5.0 -14.0	95-240+	132.5	6.7
10	Sulfaphenylthiazol	1000	7.0 -14.0	80-240+	89	10
20	None (controls)			35-240+	36.5	30
50	Sulfanilamide	1000	14.0	48-240+	87	8
10	Sulfapyridine	250	1.5 - 3.5	50-240+	132	40
20	,,	500	3.0 - 7.0	56-240+	240+	55
25	,,	1000	8.0 -14.0	90-240+	240+	52
10	Sulfathiazol	250	1.25- 3.5	32-240+	90	40
10	,,	1000	5.0 -14.0	40-240+	240+	70
10	Sulfamethylthiazol	250	1.25- 3.5	65-240+	240+	80
10	,,	500	4.0 - 7.0	96-240+	240+	80
15	,,	1000	8.0 -14.0	100-240+	240+	60

* Results of two separate tests are shown in order to illustrate the relation between virulence and therapeutic efficiency of the compounds.

effective therapy, the following study of absorption of the thiazol compounds was undertaken.*

Twenty-five mice, weighing from 20 to 24 g each, were medicated by stomach tube with 1 cc of a milk suspension of sulfapyridine in a dosage of 100 mg per kg body weight. A second group of 25 animals was medicated similarly with a dosage of 1000 mg per kg. These experiments were duplicated one or more times at each dosage level with each of the following compounds: sulfathiazol, sulfamethylthiazol, and sulfaphenylthiazol. Five mice from each dosage level for each compound were killed at 2, 4, 6, 24, and 48 hours after medication. The blood of the 5 mice was pooled at each of the above time intervals, and the amount of the free drug and total drug (free and combined) was determined by a modification of the Marshall method,[2, 3] using a new coupling reagent: N(l-naphthyl) ethylene diamine dihydrochloride.

---

* A complete report on the absorption, excretion and toxicity of the thiazol derivatives of sulfanilamide in laboratory animals and human beings will be published elsewhere.

The observed blood values at the above time intervals post medication are indicated in Table V.

In the dosage range tested, these data do not indicate the minimally effective antistreptococcal blood concentrations of these compounds. However, the effective concentrations of the several preparations are probably less than those indicated in Table V after medication with the single 100 mg/kg dosage. Under similar conditions much higher blood concentrations (3.8 to 15 mg %) were found optimal for effective therapy of experimental pneumococcal infections.

It is of interest to note that the degree of conjugation which occurs in the mouse in order from greatest to least is: sulfaphenylthiazol, sulfapyridine, sulfathiazol or sulfamethylthiazol.

Sulfaphenylthiazol is excreted by mice and other animals (cats,

TABLE V.
Blood Concentration and Degree of Conjugation (Mice).

Preparation	Sulfa-pyridine		Sulfa-thiazol		Sulfamethyl-thiazol		Sulfaphenyl-thiazol	
Oral dose, g/kg	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0
No. of mice	25	25	25	25	50	75	25	25
2 hrs after medication								
Free mg %	4.6	11.8	2.5	12.5	8.8	12.2	6.7	14.3
Total mg %	4.7	14.9	2.9	13.8	9.15	13.5	7.8	16.7
% of total conjugated	2.1	20.8	13.8	9.4	3.8	9.6	14.1	14.3
4 hrs after medication								
Free mg %	3.2	15.2	2.3	9.5	5.1	11.0	5.4	14.6
Total mg %	4.2	18.0	2.7	10.5	5.8	12.6	8.1	17.4
% of total conjugated	23.8	15.5	14.8	9.5	12.0	12.7	33.3	16.09
6 hrs after medication								
Free mg %	1.4	15.0	2.0	8.6	4.0	10.3	5.1	9.3
Total mg %	1.8	18.2	2.3	10.2	4.5	11.9	7.3	13.3
% of total conjugated	22.2	17.5	13.0	15.7	11.1	13.4	30.1	30.0
24 hrs after medication								
Free mg %	trace	3.3	—	4.1	—	2.5	0.6-0.7	3.3
Total mg %	trace	3.8	0.2	6.0	trace	4.0	2.2	6.4
% of total conjugated	—	13.1	—	31.6	—	37.5	70.0	48.4
48 hrs after medication								
Free mg %	—	—	—	—	—	trace	—	trace
Total mg %	—	0.4	—	—	—	0.8	0.9	1.2
% of total conjugated	—	—	—	—	—	—	—	—

---

[2] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

[3] Bratton, A. L., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

dogs, man—unpublished) more slowly than the other 3 compounds so that cumulation tends to occur after frequently repeated doses.

Tolerance of Single Doses. Single oral doses of 25 g/kg of sulfapyridine or of the thiazol derivatives of sulfanilamide were tolerated by groups of 25 young adult albino mice. No symptoms of any kind were noted, other than some depression due to handling and to dilatation of the stomach. This high tolerance is largely due to the relatively low absorption of these compounds. The major portions of such large doses are passed in the feces.

Sulfapyridine sodium in large oral doses to mice produces a marked increase in reflex excitability, muscular tone, opisthotonus or even severe convulsions within 3 to 10 minutes after medication. These effects persist for from 45 to 90 minutes and are terminated by death or followed by a progressive depression and an irregular labored respiration to the 2nd or 3rd hour with gradual recovery to normal by the 7th hour, as described by Marshall and Long.[4]

On the other hand, intolerance to oral doses of the sodium salts of the 3 thiazol derivatives of sulfanilamide was indicated by a marked general depression and irregular respiration corresponding to the secondary effects of sulfapyridine sodium.

Table VI permits a comparison of the single M.L.D. (60% kills) and absolute lethal oral doses of the soluble salts of the several compounds for mice. The dosages were calculated on the basis of the

TABLE VI.
Oral Toxicity of Sulfapyridine and Thiazol (as Sodium Salts) for Mice.

Dose, g/kg	Aqueous concen. used %	Sulfapyridine		Sulfaphenylthiazol		Sulfamethylthiazol		Sulfathiazol	
		No. mice	% kills	No. mice	% kills	No. mice	% kills	No. mice	% kills
1.0	10	11	0	11	0	11	0	11	0
1.5	10	11	9.09	11	0	11	0	11	0
1.75	10	11	63.6	11	9.09	11	0	11	0
2.0	10	11	72.7	11	18.18	11	0	11	0
2.25	10	11	90.9	11	36.36				
2.5	10	11	90.9	11	63.6	11	0	11	0
2.75	10	11	90.9	11	72.7				
3.0	10	11	100.0	11	81.8	11	0	11	0
3.5	10	11	100.0	11	100.0	11	0	11	9.09
4.0	10			11	100.0	11	0	11	18.18
4.5	10			11	100.0	11	0	11	18.18
5.0	10					11	9.09	11	18.18
5.5	10					11	36.36		
6.0	10					11	63.63	11	27.27
7.0	10					11	90.9	11	54.54
8.0	10					11	100.0	11	81.81
10.0	10					11	100.0	11	100.0

---

[4] Marshall, E. K., Jr., and Long, P. H., *J. A. M. A.*, 1939, **112**, 1671.

compounds *per se* and the maximal volume by tube per mouse did not exceed ½ cc. These data indicate that sulfapyridine sodium orally to mice is 1.4, 3.4 and 4.57 times more toxic than the sodium salts of sulfaphenylthiazol, sulfamethylthiazol and sulfathiazol respectively.

Tolerance—Chronic Medication. Sulfapyridine, or the thiazol derivatives, is tolerated by groups of 50 or more mice in single daily oral doses up to 1.5 g/kg over a 15-day period or 2 g/kg daily over a 10-day period without ill effects as judged by weight maintenance and growth. Necropsy of the sacrificed animals at the 30th post-medication day likewise indicated no gross abnormalities.

However, single daily doses of 5 g/kg by stomach tube to groups of 30 mice over a 10-day period resulted in a cumulative toxicity. This maximal dosage represented the M.L.D. (60% kills) of sulfapyridine, but killed only 10% of animals receiving sulfathiazol, 20% of animals to which sulfaphenylthiazol was administered and 30% of the mice which were medicated with sulfamethylthiazol. The majority of all deaths occurred after discontinuance of medication up to the 19th day. Necropsy of such animals showed parenchymatous changes of greater or less degree in the kidney. The necropsy of the surviving animals of each group sacrificed after 30 days was essentially negative.

Conclusions. The chemotherapeutic effects of 3 thiazol derivatives of sulfanilamide under conditions of experimental streptococcal or pneumococcal infections are definitely superior to those of the parent substance and compare very favorably with those of sulfapyridine. On the basis of the superior margin of safety of sulfathiazol and particularly sulfamethylthiazol, as compared with sulfanilamide and sulfapyridine, these new compounds appear quite promising.

11184

Bacteriostatic Effects of Sulfathiazole upon Various Micro-Organisms. Its Therapeutic Effects in Experimental Pneumococcal Infections.*

Perrin H. Long and Eleanor A. Bliss.

From the Biological Division, Department of Medicine, The Johns Hopkins University.

McKee and her coworkers[1] have reported briefly that sulfathiazole is bacteriostatic *in vitro*. In Table I are recorded data regarding the comparative bacteriostatic effects of sulfanilamide, sulfapyridine and

TABLE I.
Comparative Bacteriostatic Effects of Sulfanilamide, Sulfapyridine and Sulfathiazole upon Various Bacteria in Broth.

		Growth—Colonies/cc 17-24 hours			
Organism	Inoculum Orgs./cc	Sulfanilamide 10 mg %	Sulfapyridine 10 mg %	Sulfathiazole 10 mg %	Controls
Hem. Strept. Group A					
C203	42	75,000	70,000	30,000	810 million
”	108	74 million	20 million	800,000	550 ”
Bart	60	70,000	40,000	23,000	560 ”
”	66	65 million	1 million	1 million	500 ”
N.Y.5	42	80,000	90,000	123,000	590 ”
”	46	74 million	16 million	1 million	320 ”
Hem. Strept. Group D					
Zymogenes	420	2 billion	1.85 billion	1.75 billion	2.2 billion
”	8	1.07 ”	1.46 ”	1.34 ”	1.02 ”
Hem. Strept. Group G					
Dog	152	22,000	37,000	80,000	450 million
”	202	70,000	89,000	79,000	167 ”
Pneumococci					
Type I	17,200	2 million	1 million	4 million	630 million
”	280	30 ”	20,000	10,000	1.7 billion
SVI P/47	23,400	28 ”	425 million	12 million	1.35 ”
Type II	2,700	4,000	3,000	11,000	1.2 ”
”	3,800	200,000	300,000	150,000	920 million
S. aureus	12	270 million	214 million	102 million	1 billion
”	18	900 ”	510 ”	380 ”	1.8 ”
E. coli	6	1.8 billion	2.9 million	1.4 million	2.2 billion
”	80	—	70 ”	19 ”	2.5 ”
B. proteus	36	2 million	60,000	50,000	6 billion

---

* This investigation has been supported by a grant from the Chemical Foundation, Inc., of New York City.

[1] McKee, C. M., Rake, G., Greep, R. O., and Van Dyke, H. B., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 417.

sulfathiazole upon the growth of certain bacteria in beef infusion, 2% Neopeptone 0.075% dextrose broth. A perusal of the results shown in this table indicates that sulfathiazole is an effective bacteriostatic agent *in vitro,* being equal in this respect to both sulfapyridine and sulfanilamide.

It is important, in attempting to evaluate the therapeutic effectiveness of any new sulfanilamide derivative, that data regarding the absorption and excretion of the compound be available. This point was recognized by McKee, *et al.,*[1] in their report upon the comparative therapeutic value of sulfathiazole and sulfapyridine in the control of experimental infections in mice, but was not taken into consideration by Cooper, Gross and Lewis.[2] Previous studies[3, 4] have shown that sulfathiazole is absorbed more promptly and excreted more rapidly than sulfapyridine. Hence, in evaluating the comparative therapeutic effects of these two compounds when they are administered to mice by the oral route in experimental infections, the question as to whether adequate concentrations of sulfathiazole were maintained in the blood of the experimental animals is of importance. Up to the present time, all reports dealing with the comparative therapeutic effects of sulfathiazole and sulfapyridine in experimental pneumococcal infections in mice show that the latter compound is slightly more effective, *when the drugs are administered perorally in acacia suspensions at stated intervals.* As will be shown in Table II this has been our experience also. In these tests we employed 2 strains of Type I pneumococci, SVI, which is highly lethal for mice, but kills them slowly, thus making the infections quite susceptible to chemotherapy, and a strain of the Type I pneumococcus which kills mice rapidly and produces an infection in them which is very difficult to cure. The characteristics of these 2 strains in mice have been described previously.[5] As is recorded in Table II, therapy with sulfapyridine gave slightly better results in experimental infections produced in mice by the intraperitoneal injection of these 2 strains of Type I pneumococci than did sulfathiazole. This slight therapeutic superiority of sulfapyridine was more noticeable in infections produced by the strain "Pneumo I" than it was in infections produced by strain SVI. With the latter strain there was no statistical difference in the number of mice surviving after treatment, although the average survival time of the mice treated with sulfathiazole was somewhat

---

[2] Cooper, F. B., Gross, P., and Lewis, M., *Ibid.*, 1939, **42**, 421.

[3] Van Dyke, H. B., Greep, R. O., Rake, G., and McKee, C. M., *Ibid.*, 1939, **42**, 410.

[4] Long, P. H., Haviland, J. W., and Edwards, L. B., *Ibid.*, in press.

[5] Long, P. H., and Bliss, E. A., *Ann. Int. Med.*, 1939, **13**, 232.

TABLE II.
The Comparative Therapeutic Effects of Sulfapyridine and Sulfathiazole upon Experimental Pneumococcal Infections in Mice.

Organism	No. Mice	Inoculum M.L.D.	Drug	Deaths—Days after infection										Survivals	
				1	2	3	4	5	6	7	8	9	10-30	No.	%
Pneu. SVI	15	570	S.T. D30			2		1	2	2	1		1	6	40
,,	15	570	S.P. D30			1	1	1	2				2	9	60
,,	50	615	S.T. D10		1	1	1			2	3		4	38	76
,,	50	615	S.P. D10		1	1	1	2		2				43	86
,,	50	615	Controls	16	34									0	0
Pneu. I	30	600	S.T. D30	1	19	10								0	0
,,	30	600	S.P. D30		16	7	3	1					1	2	7
,,	50	710	S.T. D10	1	22	10	5	6	1	2	1			2	4
,,	50	710	S.P. D10		3	12	22	4		2	1			6	12
,,	50	710	Controls	50										0	0

S.T. = Sulfathiazole
S.P. = Sulfapyridine
D30 = 30 mg *per os* immediately after infection and 6 hours later, then Q.D. for 3 days.
D10 = 10 mg *per os* T.I.D. for 5 days, B.I.D. for 1 day, Q.D. for 1 day.

less than that of those treated with sulfapyridine. However, in the infections produced by strain "Pneumo I", therapy with sulfapyridine proved superior to that with sulfathiazole, both in relation to average survival time and to the number of mice surviving at the end of the 30-day period of observation. However, we attribute this superiority to the fact that under the conditions of our tests, the concentrations of sulfapyridine were better maintained in the blood of the mice than were those of sulfathiazole.

McKee[1] found that when sulfapyridine and sulfathiazole are administered as 1% of the diet, the therapeutic effect of the 2 drugs in experimental pneumococcal infections in mice is the same. Preliminary tests which we have carried out tend to confirm this observation. While our experience has necessarily been limited, we have noted that sulfathiazole has a definite therapeutic effect in human pneumococcal pneumonia. However, it is too early to make any comparison of the therapeutic effects of sulfathiazole and sulfapyridine in this disease.

Conclusions. Sulfathiazole is as effective a bacteriostatic agent as is sulfapyridine in broth cultures of certain strains of Lancefield's Groups A, D, and G *beta* hemolytic streptococci, *E. coli, Staphylococcus aureus,* Types I and II pneumococci and *B. proteus.* Under the conditions of our tests sulfathiazole was slighly less effective than sulfapyridine in the control of experimental pneumococcal infections in mice. However, as has been pointed out, the more rapid absorption and excretion of sulfathiazole by the mouse, partially invalidate the results of comparative therapeutic tests in which the drug is administered *per os* in acacia suspensions at stated intervals.

We wish to thank E. R. Squibb and Son and the Calco Division of the American Cyanamid Company for supplying us with sulfathiazole.

11185

Acute Toxicity, Absorption and Excretion of Sulfathiazole and Certain of its Derivatives.*

Perrin H. Long, James W. Haviland and Lydia B. Edwards.†

From the Biological Division, Department of Medicine, The Johns Hopkins University School of Medicine.

It has been repeatedly shown that the use of the peroral route of administration is not valid in assaying the acute toxicity of certain poorly soluble derivatives of sulfanilamide. Sulfathiazole[1, 2] is such a derivative and hence, in determining the acute toxicity of this drug and of its methyl and phenyl derivatives in mice, we have used the sodium salts of these compounds and have administered them by the

TABLE I.
Acute Toxicity of Sodium Salts of Sulfanilamide, Sulfapyridine, Sulfathiazole, Sulfathiazole Methyl and Sulfathiazole Phenyl When Injected by Parenteral Route in Mice.

Compound		Dose g/kilo mouse subcutaneous	No. of mice	No. dead	% dead
Sodium	Sulfanilamide	3.0	25	11	44
”	Sulfapyridine	0.5	15	1	7
		1.0	14	7	50
		1.5	14	13	93
”	Sulfathiazole	0.5	25	3	12
		1.0	25	3	12
		1.5	25	6	24
		1.75	25	6	24
		2.0	40	17	42
		2.25	25	20	80
		2.5	10	10	100
”	Sulfathiazole Methyl	0.5	25	1	4
		0.75	25	17	68
		1.0	25	20	80
		1.5	10	9	90
”	Sulfathiazole Phenyl	0.5	25	5	20
		0.75	25	7	28
		1.0	25	18	72
		1.5	10	9	90
		2.0	10	10	100

---

* This investigation has been supported by a grant from the Chemical Foundation, Inc., of New York City.

† Fellowship in medicine supported by E. R. Squibb and Son.

[1] Fosbinder, R. J., and Walter, L. A., *J. Am. Chem. Soc.*, 1939, **61**, 2032.

[2] Van Dyke, H. B., Greep, R. O., Rake, G., and McKee, C. M., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 410.

TABLE II.
Blood Concentrations of Sulfathiazole Following Single Peroral Doses of the Drug.

		Blood concentrations mg%, hr following administration of sulfathiazole													
		1		2		4		8		12		24		48	
Species	Dose, g/kilo	F	T	F	T	F	T	F	T	F	T	F	T	F	T
Mouse	0.5	10.2	11.09	9.8	11.0	3.4	4.2	1.8	2.2			S.Tr	Tr		
,,	1.0	17.5	17.8	8.0	8.6	9.6	10.2	4.0	5.6			S.Tr	Tr		
Man	0.05	6.3	7.3			6.3	7.3	3.2	5.3	2.2	4.0	S.Tr	1.6		
,,	0.05	3.3	4.3			8.2	8.6	3.6	5.0	2.5	3.1	S Tr	1.5		
,,	0.05	4.8	5.9	5.9	7.2	4.6	5.7	2.3	4.2	Tr	1.3	S.Tr	S.Tr		
Child	0.05	3.5	4.6			3.0	4.5	1.3	2.2			S.Tr	Tr		
,,	0.05	2.7	3.3			3.1	3.8	1.5	2.1			S.Tr	Tr		
,,	0.05			4.7	4.8	3.3	3.6	0.9	2.1			S.Tr	Tr		
,,	0.05	5.6	6.3			3.4	4.1	1.3	2.1			S.Tr	Tr		

F = Free Sulfathiazole
T = Total Sulfathiazole
Tr = Trace
S.Tr = Slight trace

parenteral route. Solutions of the sodium salts of sulfanilamide, sulfapyridine and of sulfathiazole and its derivatives in distilled water are very alkaline (pH 10-11) and, when they are absorbed into the blood and tissues, the sodium ion is split off, leaving the parent compound. Hence, by employing the sodium derivatives, it is possible to determine with accuracy the true toxicity of the poorly soluble parent drug.

In Table I are recorded data dealing with the acute toxicities of these compounds in mice. It is noteworthy that sulfathiazole, in comparison with sulfapyridine, has a relatively low degree of toxicity. Using the data presented in Table I we have calculated the LD_{50} for the sodium salts of sulfapyridine, sulfathiazole, sulfathiazole methyl and sulfathiazole phenyl. The LD_{50} for sodium sulfapyridine is 1.0 g per kilo with a standard error of ±.08, for sodium sulfathiazole 1.95 g per kilo with a standard error of ±.08, for sodium sulfathiazole methyl 0.86 g per kilo with a standard error of ±.06, and for sodium sulfathiazole phenyl 0.9 g per kilo with a standard error of ±.08. These results show, therefore, that the introduction of the methyl and phenyl groups increases the acute toxicity of sulfathiazole for mice by more than 50%.

It has been shown before that unless data regarding the absorption and excretion of new sulfanilamide derivatives are available, the interpretation of experimental therapeutic results and the clinical use of such new compounds is difficult. In Table II are given data regarding the concentrations of sulfathiazole noted in the blood when the drug is administered by the oral route to mice and human beings. It is of interest that the curves of the concentrations of sulfathiazole in the blood of mice following a single peroral dose of the drug, more closely resemble those previously observed for comparable doses of sulfanilamide than those noted from single doses of sulfapyridine. The drug is absorbed more readily and its concentration in the blood decreases more rapidly than does that previously noted for sulfapyridine. While it is impossible to judge accurately the degree of acetylation of the drug in the tissues of human beings from single dose experiments, our experience in the use of sulfathiazole in about 50 patients leads us to believe that its percentage of conjugation in the tissues is somewhat less than that noted for sulfapyridine. These observations are in harmony with those made by Van Dyke, *et al.*,[2] upon monkeys.

These latter observers[2] have reported that the degree of acetylation of sulfathiazole in the urine of rats and monkeys is definitely less than that of sulfapyridine. Our studies upon the excretion of this

TABLE III.

Urinary Excretion of Sulfathiazole Following Administration of Single and Repeated Doses of the Drug.

Subject	Dose of drug	Urine Volume, Excretion in mg %—Days									Total drug excreted g	% of dose excreted
		1			2			3				
		U.V.cc	F	T	U.V.cc	F	T	U.V.cc	F	T		
J.T.	Single 0.05 g/kilo Total = 3.7	760	155	191	435	30	62.5				1.7	46
L.	Single 0.05 g/kilo Total = 3.7	1550	107	235	980	S.Tr	Tr				3.64	98
P.	Single 0.05 g/kilo Total = 2.8	1500	71.5	83.7	750	8.5	13.25				2.25	80
W.L.	Single 0.05 g/kilo Total = 1.7	1640	69	100	1700	S.Tr	Tr				1.64	96
B.W.	Single 0.05 g/kilo Total = 1.8	1300	65.5	116	2360	Tr	9.1				1.72	95
W	6.0 per day	980	338	443	910	296	346				8.45	70

U.V. = Urine volume
F = Free sulfathiazole
T = Total sulfathiazole
Tr = Trace
S.Tr = Slight trace

compound in human subjects confirm these observations. In Table III are presented data bearing upon the excretion of sulfathiazole in the urine of human beings. At once, one is struck by the fact that in comparison with sulfapyridine, definitely less of the excreted sulfathiazole is present in the conjugated form. It is also interesting that the excretion of this compound, following a single oral dose, is generally complete within 24 hours, and that following such a dose, from 80% to 90% of the drug was recovered in the urine, thus denoting that the absorption of sulfathiazole under conditions of the test, was much more complete than one would expect had the subject been given sulfapyridine.

Conclusions. It has been shown that the acute toxicity of sulfathiazole (as measured by the parenteral injection of sodium salts) for mice is one-third greater than that of sulfanilamide, and about half that of sulfapyridine, sulfathiazole methyl and sulfathiazole phenyl. Sulfathiazole is absorbed more readily and is excreted more rapidly than is sulfapyridine. Because of its rate of excretion it is probable that doses of sulfathiazole spaced at intervals of 4 hours will maintain adequate concentrations of the drug in the blood of patients.

We wish to thank E. R. Squibb and Son and the Calco Division of the American Cyanamid Company for supplying us with sulfathiazole, and the Department of Medical Research of the Winthrop Chemical Company, Inc., for supplying the sulfathiazole methyl and the sodium salt of sulfathiazole phenyl.

11186 P

Agent of Lymphogranuloma Venereum in the Yolk-Sac of the Developing Chick Embryo.

GEOFFREY RAKE, CLARA M. MCKEE AND MORRIS F. SHAFFER.
From the Squibb Institute for Medical Research, New Brunswick, N. J.

In the course of chemotherapeutic studies, experiments were performed with a strain of the agent of lymphogranuloma venereum* in which passage-mouse brain was used as a source of virus. About one-third of all mice given intracerebrally 0.03 ml of 1:100 dilution died. The L_D50[1] was obtained with a dilution of 1:14. Because of its low mouse-virulence, which is in confirmation of the results of others,[2,3] an attempt was made to adapt the infective agent to the chorio-allantois of the chick embryo[4] in the hope of increased activity. Lesions appeared on this membrane, albeit irregularly.[5] Thus during 15 passages, approximately two-fifths of all eggs inoculated showed no lesions; one-fifth showed large central foci on the membrane; and in two-fifths the chorio-allantois showed few or many grey foci, 1 mm in diameter. The virus on the chorio-allantois was never lethal for the embryo. When 3rd, 4th and 5th passage membranes were tested in mice, 3 out of 4, 0 out of 4, and 1 out of 4 animals respectively died following intracerebral infection with 1:5 dilution. No mice succumbed when inoculated from the 10 subsequent passages. It appears, therefore, that during propagation in this tissue the particular strain of lymphogranuloma venereum virus either persisted there in very low titer or else suffered a further loss in its virulence for mice.

---

* Obtained through the courtesy of Dr. Wm. L. Fleming, the School of Hygiene and Public Health, Johns Hopkins University.

[1] Reed, L. H., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

In view of these conditions, a further effort was made to procure more active infection by recourse to inoculation into the yolk-sac, a method used by Cox[6] to obtain large numbers of rickettsiae. Passages were initiated with mouse-brain material. Five, 6- or 7-day eggs were injected in the yolk-sac with a volume of 0.5 to 1 ml of suspension. In 4 passages in which inoculation was made into the yolk-sac, of the various materials (brain, viscera, chorio-allantois, yolk-sac, yolk and vitelline) which were separately tested after 3 or 4 days' incubation of the egg, virus was demonstrated only in the yolk-sac material and undiluted yolk. From the 5th passage onward only yolk-sac material was used as inoculum. In this passage, eggs receiving 1:15 dilution of yolk-sac were dead by the 4th day and mice died after infection with a 1:100 dilution. Smears made from infected yolk-sacs showed very numerous minute granules which stained with Giemsa stain. These granules, never seen in preparations of normal yolk-sac, resembled the "granulo-corpuscles" seen in the meningeal exudate of mononuclear cells in mice infected with lymphogranuloma venereum[7,8] and were similar to the elementary

---

[2] Levaditi, C., Ravaut, P., Schoen, R., and Levaditi, J., *C. R. Soc. Biol.*, 1933, **114**, 499.

[3] MacCallum, F. O., and Findlay, G. M., *Lancet*, 1938, **2**, 136.

[4] Burnet, F. M., *Med. Res. Counc. Spec. Rep.*, Ser. No. 220, London, 1936.

[5] Sanders, M., *Arch. Path.*, 1939, **28**, 541.

[6] Cox, H. R., *Pub. Health Reports*, 1938, **53**, 2241.

[7] Miyagawa, Y., Mitamura, T., Yaoi, H., Ishii, N., Nakajima, H., Okanishi, J., Watanabe, S., and Sato, K., *Jap. J. Exp. Med.*, 1935, **13**, 733.

[8] Schoen, R., *Ann. Inst. Pasteur*, 1939, **62**, 260.

bodies of vaccinia. In smears the granules were seen chiefly lying free although they were occasionally observed within cells. In sections the granules were found to be chiefly intracellular, lying in large numbers within the yolk-sac cells. They are probably liberated into the surrounding yolk by disruption of these cells.

Over 25 yolk-sac passages have been completed. The infectious agent is now lethal within 9 days for the developing embryo in dilutions through 10^{-9}. A typical titration at the 20th passage is shown (Table I).

TABLE I.

Dilution of yolk-sac	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
No. of days' infection to death of embryo	7	6,7	7,7	7,8	8,8	9,9

While careful titrations were not carried out in early passages, the virulence of the virus for the embryo does not appear to have increased greatly. At the present time dilutions of 1:10 require 3 or 4 days to kill as they did at first. During the course of the 25 yolk-sac-passages about 1 mouse in 5, of those receiving 1:1000 dilution of material, died and the $\mathrm{L_D}50$ for mice was obtained with a dilution of 1:200.

The granules were purified by differential centrifugation in the cold. The material was spun for 1 hour at 2000 rpm; the sediment was discarded and the supernate recentrifuged for 2 hours at 12,000 rpm. The sediment from this second centrifugation represented a preparation freed from most yolk-sac constituents and contained a rich suspension of the granules which are believed to be the elementary bodies of the infectious agent. Several tests of the sediment and supernate after high speed centrifugation showed nearly all of the mouse-infectious material to be in the sediment. The small amounts found in the supernate were believed to be due to insufficient centrifugation.

Both formalinized and untreated suspensions of such granules evoke specific agglutinins in rabbits (serum titers of 1:400) and, to a lesser degree, in chickens.

We believe that the use of the yolk-sac technic offers a method of great promise in the further experimental study of the agent of lymphogranuloma venereum, as well as in the development of improved reagents for diagnosis, prophylaxis and therapy (*e. g.,* Frei antigen and hyper-immune serum).

11187 P

Effect of Magnesium Sulfate on Serum and Peritoneal Fluid Calcium, Magnesium, Inorganic Phosphorus.*

V. G. Haury and A. Cantarow.

From the Departments of Pharmacology and Medicine, Jefferson Medical College, and the Laboratory of Biochemistry, Jefferson Hospital, Philadelphia.

Four adult dogs were given intraperitoneal injections of 2.5% dextrose and 0.9% NaCl solution in the dosage of 100 cc per kg. After 16 hours, 20 cc of blood were withdrawn from the femoral artery and 20 cc of fluid from the peritoneum. Previous observations[1] have revealed that the serum and peritoneal fluid Ca, Mg and P are in equilibrium at this time. Fifty cc of a 25% solution of $MgSO_4$ were injected intramuscularly (3 cases) or subcutaneously (1 case). Blood and fluid samples (20 cc each) were obtained ½, 1, 1½, 2, 3, and 4½ hours after injection of the $MgSO_4$, and were examined for their Mg, Ca and inorganic P content.

Magnesium. The control values for serum Mg were 1.82-2.46 mg %. The maximum values after injection of magnesium ranged from 8.9 (subcutaneous injection) to 16.1 mg %, the peak being reached in 1-2 hours, with a subsequent decline.

The control values for peritoneal fluid Mg were 1.5-1.67 mg %. These increased in each instance, to values of 6.4-12.2 mg %, the peak being reached ½-1½ hours after the peak of serum Mg concentration.

Calcium. The control serum calcium values were 9.45-12.1 mg %. These fell in each instance, to minimum levels of 6.39, 7.0, 7.6 and 8.92 mg %, the last in the animal injected subcutaneously. The maximum drop in serum Ca concentration (2.31-4.5 mg %) occurred ½-3½ hours after the peak of serum Mg concentration.

The control values for peritoneal fluid Ca were 5.94-8.5 mg %. These changed relatively slightly during the experimental period, the range of maximum variation from the control values being +0.63 to – 1.2 mg %, the latter occurring in the case in which the serum Ca concentration fell 4.5 mg %.

Inorganic Phosphorus. In 2 of the 3 cases in which this determination was made the serum inorganic P concentration fell from con-

---

* Aided by a grant from Parke, Davis and Co.

[1] Cantarow, A., Haury, V. G., and Whitbeck, C. G., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 15, 18; Cantarow, A., and Haury, V. G., *Am. J. Physiol.*, 1939, **126**, 66.

trol levels of 4.6 and 6.63 mg % to minimum levels of 3.1 and 3.54 mg % respectively, 1 and 1½ hours after the peak of serum Mg concentration had been reached. A similar fall occurred in the peritoneal fluid P concentration in these cases, from control values of 4.4 and 6.36 mg % to minimum values of 3.0 and 3.6 mg %. In the other case there was no significant change in serum or peritoneal fluid P concentration.

Comment. The fall in serum Ca following injection of $MgSO_4$ is in accord with previous observations,[2-5] some of which suggest that the SO_4 ion participates in the production of this effect. Meneghetti[4] reported a simultaneous increase in diffusible Ca (rabbits), a finding not in accord with those reported here. The observed fall in serum inorganic P has also been reported previously,[2, 6] being perhaps also dependent in part on the influence of the SO_4 ion. The simultaneous fall in diffused P (peritoneal fluid), not reported previously, is particularly significant in view of the relatively insignificant alteration in peritoneal fluid Ca concentration. It may be related to the simultaneous increase in the concentration of Mg and perhaps SO_4 in the fluid.

It is of interest that frank tetany (relieved promptly by intravenous injection of calcium glucogalactogluconate) occurred in the 3 animals in which the serum Ca concentration fell below 8 mg % despite the absence of significant alteration in the peritoneal fluid Ca concentration in 2 of these animals. The explanation of this phenomenon is not readily apparent. If, as suggested by previous reports from this laboratory,[1] the peritoneal fluid Ca concentration may be regarded as representative of the concentration of Ca in the tissue fluids, the significance of the latter in hypocalcemic tetany appears to be open to question. It is difficult, too, to understand the occurrence of tetany with relatively slight degrees of hypocalcemia in the presence of such marked increase in the Mg concentration of both serum and peritoneal fluid (and, presumably, tissue fluids).

---

[2] Brookfield, R. W., *Biochem. J.*, 1934, **28**, 725.

[3] Stransky, E., *Arch. exp. Path. u. Pharmakol.*, 1915, **78**, 122.

[4] Menenghetti, E., *Biochim. e terap. sper.*, 1927, **14**, 116.

[5] Pribyl, E., *Compt. rend. Soc. de Biol.*, 1929, **102**, 258.

[6] Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, 1935, **15**, 297.

11188 P

Intranasal Inoculation of Human Individuals with the Virus of Epidemic Influenza.*

THOMAS FRANCIS, JR.

From the Department of Bacteriology, New York University College of Medicine.

Burnet and Lush have reported the intranasal inoculation of a series of human individuals with a strain of influenza virus propagated on the membrane of the developing egg.[1] The strain, so maintained, had lost its capacity to elicit clinical infection in ferrets and was of extremely low virulence for mice. Of the human subjects tested none showed evidence of infection. Subsequent to this exposure, the serum of none of 18 subjects examined exhibited a rise in circulating antibodies, as measured by the mouse protection test, while a minority of them showed some increase when the sera were titrated with egg passage virus on the egg membrane.

The use of the nasal route for vaccination against epidemic influenza offers several theoretical advantages as well as disadvantages. Among the former are the fact that the nasal route represents the normal route of infection and a subclinical infection so produced might result not only in antibody response to an actual, mild infection but in a protective conditioning of the respiratory membranes as well. On the other hand, the most obvious objection is the possibility of establishing infection which could be transmitted from one individual to another. It seemed likely that the essentially negative results previously mentioned were attributable to the use of an avirulent strain of virus. It was desirable, therefore, to undertake similar experiments with human subjects employing virus of known animal pathogenicity, the virulence of which could be readily titrated.

The PR8 strain of the virus of epidemic influenza had been maintained through 480 transfers in tissue culture medium for over 4 years at the time the experiments were undertaken. When tested in ferrets, the undiluted culture induced fever and nasal discharge but no pulmonary lesions. When diluted tenfold, the virus caused no elevation of temperature above the normal limit of 104°F in the ferret, nasal signs were slight, but the animal developed a high titer of neutralizing antibodies. In mice the culture of virus usually produced fatal infection when 0.05 cc of a 1:1000 dilution was given

---

* This study was conducted under a grant from the International Health Division of the Rockefeller Foundation.

[1] Burnet, F. M., and Lush, D., *Brit. J. Exp. Path.*, 1938, **19**, 17.

TABLE I.

Neutralizing Titer of Serum of Human Subjects Before and After Intranasal Inoculation of the Virus of Influenza.

Neutralizing titer of serum	Test Subject										
	I.B.	H.M.	W.V.	C.S.	A.M.	D.C.	E.H.	M.L.	D.R.	M.S.	L.W.
Before inoculation	1:35	1:140	1:120	1:140	0	1:15	1:70	1:17.5	1:60	1:60	1:4
After ”	1:35	1:140	1:120	1:140	1:15	1:17.5	1:100	1:15	1:60	1:50	0

intranasally and pulmonary lesions were produced by culture diluted 1:10,000.

Eleven healthy human subjects with no history of immediately recent respiratory infection were selected. Throat cultures had been made to detect any abnormal pharyngeal bacterial flora. Serum was obtained before inoculation. Fresh cultures of virus were prepared and mice were inoculated intranasally at the time of their use. The culture was diluted tenfold and with the head tilted backward, 0.25 cc of the dilution, containing approximately 500 fatal doses for mice, was allowed to run into each nostril of the human subject from a pipette. Little or none escaped into the pharynx. The head was then thrown forward. Daily leukocyte counts were done on the first 5 subjects and in all, temperatures were taken twice daily and suggestive symptoms were noted for a period of a week. Two weeks after the inoculation serum was again obtained and titrations of the content of neutralizing antibodies of both sera were done simultaneously by the method of Francis and Magill.

One subject had a rise of temperature to 99°, p.o., 30 hours after the inoculation, at which time a feeling of warmth and slight achiness was noted. One other developed an acute rhinitis without fever 8 hours after inoculation and this persisted as a common cold. A third subject complained of slight dryness in the nasopharynx on the first day after injection and on the second day labial herpes was noted. This was thought to be related to sunburn. All others remained asymptomatic and afebrile. Furthermore, titrations of the antibodies present in the sera before and after inoculation revealed that only one, the last subject mentioned above (A.M.), showed any significant elevation in antibody titer and this individual possessed no neutralizing antibodies before inoculation. Studies to be reported elsewhere regarding the inactivation of virus by nasal secretions offer an explanation for these results.

Summary. Of 11 human subjects inoculated intranasally with active cultures of the virus of epidemic influenza only one showed a rise of antibodies following the injection. None exhibited significant signs or symptoms of infection. The possible use of this procedure for preventive immunization will be further investigated.

11189 P

Effect of Stilboestrol on Certain Phases of Carbohydrate Metabolism.*

R. G. Janes and W. O. Nelson.

From the Department of Anatomy, Wayne University College of Medicine, Detroit.

Carbohydrate levels in intact animals are affected not only by insulin, anterior pituitary extracts, and adrenal-cortical-hormone, but apparently also by certain sex hormones. Zunz and LaBarre[1] found an increase in blood sugar after the administration of estradiol. Gilder and Phillips[2] showed that rats treated with this hormone had higher levels of liver glycogen when glucose-fed than did normal animals. Engelhart and Riml[3] found that the injection of corpus luteal extracts in guinea pigs increased liver glycogen. More recently, Gaunt, Remington, and Edelmann[4] administered progesterone to intact ferrets in acute experiments and found that the carbohydrate levels were elevated. However, progesterone, testosterone, or stilboestrol were without effect. Because of the potency of stilboestrol as an estrogen, we have studied its effect on carbohydrate levels after longer periods of administration.

Rats weighing from 150 to 200 g, when treatment began, received 0.05 mg of stilboestrol† subcutaneously twice daily for periods of 5, 10, and 20 days. Determinations were made for blood sugar, liver- and muscle-glycogen, and urinary nitrogen, and the gonads, adrenals, and pituitaries were weighed and studied histologically.

The blood sugar level was found to be raised somewhat after only 5 days of treatment, and considerably more after 20 days. The liver glycogen was increased markedly even after 5 days of injections, and the muscle glycogen showed a slight drop after 20 days of treatment. The latter is of doubtful significance. The urinary nitrogen, determined for the last 24 hours of a 36-hour fast, was quite variable, Since it was not markedly altered in animals that were treated for

---

* This work was assisted by grants from the American Association for the Advancement of Science and the Committee on Research in Endocrinology of the National Research Council.

[1] Zunz, E., and LaBarre, J., *Arch. Int. de Physiol.* (Liege), 1939, **48**, 287.

[2] Gilder, H., and Phillips, R. A., *Proc. Am. Physiol. Soc.*, 1939, 86.

[3] Engelhart, E., and Riml, O., *Klin. Wchnschr.*, 1934, **13**, 101.

[4] Gaunt, R., Remington, J. W., and Edelmann, A., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 429.

† Stilboestrol was generously supplied by Dr. J. A. Morrell of E. R. Squibb and Sons.

TABLE I.
Chemical and Organic Changes in Rats Receiving 0.1 mg Stilboestrol Daily (averages).

Days treated	No. of animals	Wt at end of 36-hr fast g	Blood sugar, mg %	Glycogen		Urinary N last 24 hr, mg/g	Wt adrenals, (2) g	Wt ovaries, (2) g	Wt testes, (2) g	Wt pituitary, g
				Liver, mg%	Muscle, mg %					
Control	9	170	68 ± 4†	75	310	.802	.0414	.0655	2.257	.0077
5	6	169	73 ± 12†	231	354	.774	.0626	.0820	2.330	.0116
10	10	149	74 ± 8†	331	311	.774	.0520	(3)* .0800	(2)* 1.671	.0124
20	8	131	84 ± 6†	409	260	1.042	.0720	(6)* .0780	(4)*	.0144

* Numbers in parentheses are number of animals.

† Standard deviation.

5 or 10 days, it is possible that gluconeogenesis from fat produced the increased carbohydrate levels. However, gluconeogenesis from protein might account for the heightened levels of carbohydrate in animals treated with stilboestrol for 20 days since the urinary nitrogens were increased significantly.

The effect of stilboestrol on the endocrine glands is significant. The estrogen apparently stimulates the release of hypophyseal luteinizing hormone and inhibits the production of follicle-stimulating hormone, since the ovaries showed large corpora lutea and small follicles. The adrenals were increased in size, particularly in the females, and the entire male reproductive system was damaged. In these respects stilboestrol duplicates the response obtained with natural estrogens.

The question arises, of course, as to the mechanism whereby these effects on carbohydrate metabolism are produced. The stilboestrol action may be through the adrenals, the gonads, or both. Adrenal cortical hormone will raise the carbohydrate levels in intact animals[5] in a manner similar to that described here for stilboestrol. Consequently, one might suspect that the effect obtained is through the adrenals. This is substantiated by the work of Zunz and LaBarre, who found that blood sugar levels were not raised in dogs under estradiol treatment after the adrenals were tied off. On the other hand, Gaunt, *et al.*,[4] found that progesterone was effective in producing the elevation of carbohydrates in the adrenalectomized ferret. It seems likely that increased production of progesterone is not the primary factor in causing the changes described here since stilboestrol is effective in males as well as in females. Until further studies are completed no conclusions can be drawn concerning the exact manner in which stilboestrol effects these changes in the carbohydrate levels.

---

[5] Katzin, B., and Long, C. N. H., *Proc. Am. Physiol. Soc.*, 1939, 135.

11190 P

Activity of Progesterone in Spayed Females Not Pretreated with Estrin.*

HANS SELYE.

From the Department of Anatomy, McGill University, Montreal, Canada.

Five female albino rats weighing 108-141 g were ovariectomized and beginning 2 days later received 10 daily injections of 15 mg of progesterone in 0.5 cc of peanut oil subcutaneously. At autopsy, 24 hours after the last injection, all animals showed marked mammary gland development with lobule formation. The uteri were hyperemic and enlarged and upon histological examination showed progestational changes. These findings appeared to indicate that if a sufficiently large dose of progesterone is given, the hormone elicits its characteristic effects in the absence of the ovary and without estrin pretreatment.

In order to establish whether this response to progesterone was merely due to the fact that treatment was begun soon after ovariectomy, the experiment was repeated on 3 albino females weighing 143-155 g. The animals had been spayed 9 days prior to the initiation of daily treatment with 15 mg of progesterone as in the previous series. On the tenth day of treatment, biopsy specimens of the uterus showed progestational changes while the mammary glands exhibited only slight development. One uterine horn was traumatized at the occasion of the biopsy and treatment was continued for 5 more days. Autopsy at the end of this period revealed the formation of deciduomata in the traumatized uterine horn, full development of the mammary glands and mucoid transformation of the vaginal epithelium, such as is seen in late pregnancy. The fact that in this series, the mammary glands were not well developed as yet by the tenth day indicates that in animals spayed some time before the initiation of hormone administration, a longer period of treatment is necessary in order to obtain mammary proliferation than in animals in which treatment is initiated soon after ablation of the gonad.

Previous experiments in which doses up to 5 mg of progesterone were administered to spayed rats led to the conclusion that without estrin pretreatment, the hormone of the corpus luteum exerts no

---

* The expenses of this investigation were defrayed in part by a grant received from the Schering Corporation of Bloomfield, N. J., through the courtesy of Dr. G. Stragnell. The progesterone was kindly supplied by Dr. E. Schwenk of the same corporation.

action on the sexual organs.[1, 2] In intact females, treatment with 4 mg of progesterone daily proved equally ineffective.[3] In the light of the present experiments, it appears, however, that the negative results previously reported were merely due to the administration of insufficient doses of the hormone, and that if suitable amounts are administered progesterone exerts all its characteristic actions without sensitization by estrogens.

Summary. Experiments in spayed rats indicate that daily administration of 15 mg of progesterone produces progestational changes in the endometrium vaginal mucification and mammary gland development similar to that seen in late pregnancy. It is concluded that, contrary to the generally accepted conception, progesterone can exert all its characteristic actions in spayed females without sensitization by estrogens.

11191 P

Effect of Sulfapyridine Upon Growth, *in Vitro*, of Tubercle Bacillus.

Henry Green. (Introduced by A. J. Goldforb.)

From the Department of Bacteriology, Sea View Hospital, N. Y.

The purpose of this investigation was to determine the effect, if any, exerted upon the growth of the tubercle bacillus of varying concentrations of sulfapyridine.

The culture medium employed was a meat broth prepared from fresh meat and adjusted to a pH 7.2-7.6. Glycerol was added to make a 4% solution. The media was put in flasks and sterilized in the autoclave for 20 minutes at 15 pounds per square inch pressure. After the flasks were cooled, weighed amounts of sulfapyridine were added. Since sulfapyridine is soluble only in dilutions of 1-1000 and less, dilutions of the following concentrations were made: 1-1000, 1-2000, 1-4000, 1-6000, and 1-10,000. After the addition of the sulfapyridine, the flasks were immersed in a water bath for 30 minutes at 65°C. The flasks were then ready for inoculation.

---

[1] Astwood, E. B., Geschickter, C. F., and Rausch, E. O., *Am. J. Anat.*, 1937, **61**, 373.

[2] Astwood, E. B., and Geschickter, C. F., *Arch. Surg.*, 1938, **36**, 672.

[3] Selye, H., Browne, J. S. L., and Collip, J. B., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 472.

Inoculation was performed by floating a single loop of surface growth from a stock float culture of human tubercle bacilli carried in the laboratory.

A control flask of glycerol broth, prepared and carried exactly as noted above, was utilized.

After inoculation, the flasks were incubated at 37°C.

The experiment was performed 3 times with the findings as noted in the accompanying graphs.

From these it can be seen that there is no growth in concentrations of sulfapyridine of 1-1000 and 1-2000; definite retardation of growth in 1-4000; and a slight or no retardation in growth in concentrations of 1-6000 and 1-10,000.

Besides absence of growth in concentrations of 1-1000 and 1-2000, it was noted that the physical characteristics of the float changed from its usual "healthy" dry, creamy yellow appearance to become brown and moist.

In order to determine whether the organisms were killed or merely inhibited in the concentrations of 1-1000 and 1-2000, the floats in these concentrations were removed from the culture media at the end of the experiment, washed in sterile normal saline and then planted on solid slants of standard gentian violet-egg media and incubated. In no instance was growth noted at the end of one month.

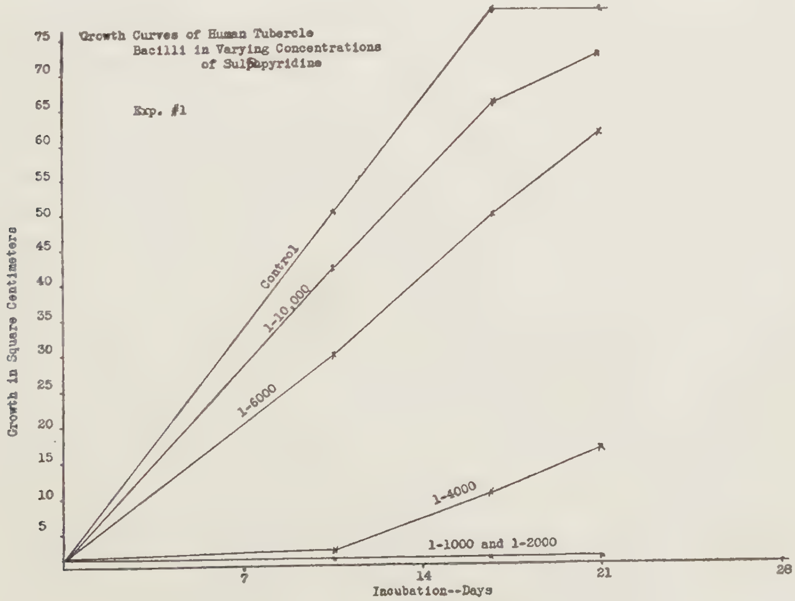


FIG. 1.

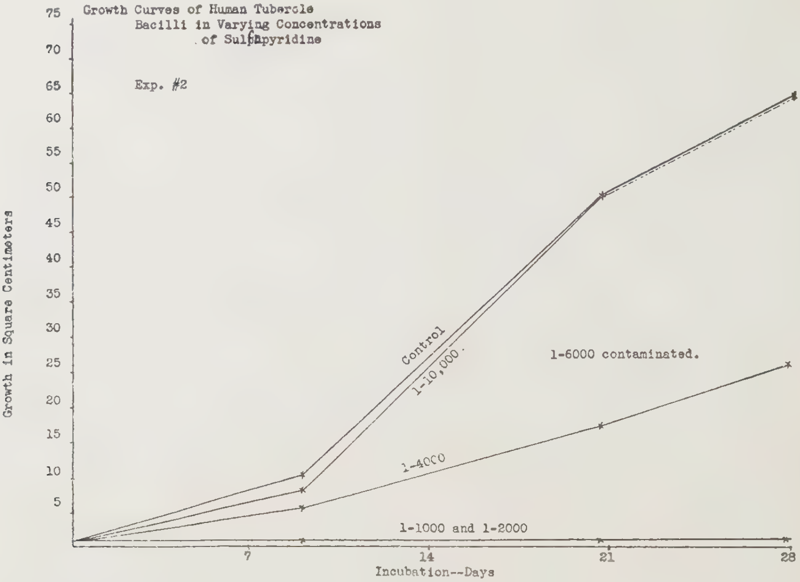


FIG. 2.

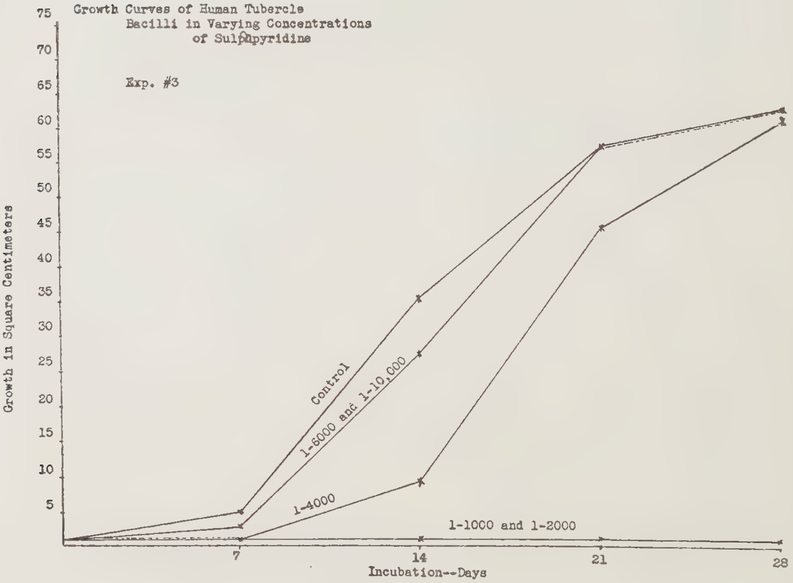


FIG. 3.

Conclusions. Under the conditions of the experiment, sulfapyridine in concentrations of 1-1000 and 1-2000 allows no growth of human tubercle bacilli. At the end of 3 to 4 weeks of incubation, the floats from these concentrations do not grow when planted on suitable media. Sulfapyridine in concentrations of 1-4000 definitely retards the growth of human tubercle bacilli. Sulfapyridine in concentrations of 1-6000 and 1-10000 affects little, or not at all, the growth of the organism.

We wish to express our appreciation to Dr. S. A. Petroff for his valuable advice.

11192 P

Insulin Hypoglycemia and Epinephrine Output from the Adrenal Glands.

J. M. Rogoff and E. Nola Nixon.

From the Laboratory of Experimental Endocrinology, School of Medicine, University of Pittsburgh.*

It has been shown that the severity of experimental pancreatic diabetes is not modified by reduction or suppression of the epinephrine output from the adrenal glands.[1] The insulin requirement under these conditions is not different from that of diabetic animals whose epinephrine secretion is not interfered with. Furthermore, it was found that depancreatized dogs, not subjected to interference with the adrenals, sooner or later develop reduced or suppressed secretion of epinephrine, which appears to be due primarily to the diabetic state.[2]

Our experimental investigations in progress have yielded results which indicate that epinephrine, when introduced intravenously at a constant rate in amounts corresponding to the ordinary rate of liberation from the adrenals in normal dogs, can elevate the level of blood sugar. The influence of similar amounts of epinephrine in animals previously subjected to operations for reduction or suppression of secretion from the adrenals is under investigation.

Although epinephrine secretion is not concerned primarily with the cause or progress of experimental pancreatic diabetes, it might, nevertheless, play a rôle, perhaps not indispensable, in normal carbohydrate metabolism. Cannon, McIver and Bliss[3] have suggested that epinephrine secretion from the adrenals constitutes a mechanism for mobilizing sugar in hypoglycemia. Their experimental evidence is in support of the view that as the level of blood-sugar declines to a "critical point" (between 110 and 70 mg % in cats, under chloralose anesthesia), an increased discharge of epinephrine from the adrenals is produced. They employed the Folin-Wu method for estimating blood sugar.

---

* Supported by the G. N. Stewart Memorial Fund, founded by contributions from the Louis D. Beaumont Trust, N. L. Dauby, Mrs. Frances W. Lang, and Howard E. Wise.

[1] Rogoff, J. M., and Ferrill, H. Ward, *Arch. Int. Med.*, 1937, **60**, 805.

[2] Rogoff, J. M., and Nixon, E. Nola, *Am. J. Physiol.*, 1937, **120**, 440.

[3] Cannon, W. B., McIver, M. A., and Bliss, S. W., *Am. J. Physiol.*, 1924, **69**, 46.

Since prolonged hyperglycemia can lead to reduction or suppression of epinephrine secretion, it appears probable that hypoglycemia might result in an increase in epinephrine output from the adrenals. Cannon, McIver and Bliss did not make quantitative measurements of the epinephrine output. They regarded acceleration of the so-called "denervated heart" as an indication of increased epinephrine discharge. Their conclusions rest on the observation that acceleration of the denervated heart did not occur at the critical point, following administration of insulin, if epinephrine secretion from the adrenals was interfered with.

Stewart and Rogoff[4] found no increase in epinephrine output, by direct measurement of the rate of liberation of epinephrine from the adrenals, in three cats under the influence of insulin. Blood sugar was estimated by the well-known Folin-Wu and the Shaffer-Hartmann methods, with good agreement. Since, in those experiments the blood sugar level did not decline to the aforementioned critical point, nor to a "hypoglycemic" level, we have performed similar experiments with the same method allowing a sufficient period for development of more marked insulin hypoglycemia. In addition, we have also performed experiments on animals with denervated heart. In these experiments the Shaffer-Hartmann method for blood-sugar was employed.

Quantitative determination of the rate of liberation of epinephrine from the adrenals was made on 2 cats and 2 dogs, after administration of effective doses of insulin. In one cat (nembutal anesthesia) the blood-sugar level remained high, in the other it declined to 36 mg %. In the 2 dogs it reached 40 mg and 37 mg %, respectively. Epinephrine output was determined at 0.00011 mg and 0.00022 mg per kg per minute for the cats, and 0.00018 mg and 0.0003 mg per kg per minute for the dogs. In each case the output was not different from that generally observed in normal animals, without insulin, under comparable experimental conditions.[5]

In the experiments on animals with the heart denervated by excision of the stellate ganglia and section of the vagi, some of our results were comparable with those reported by Cannon, McIver and Bliss. Others, however, demonstrated that it is possible to obtain excellent acceleration of the heart, following effective doses of insulin, in animals with one adrenal excised and the other denervated.

---

[4] Stewart, G. N., and Rogoff, J. M., *Am. J. Physiol.*, 1923, **65**, 331.

[5] Stewart, G. N., and Rogoff, J. M., *Am. J. Physiol.*, 1923, **66**, 235.

In one of the experiments, which in our opinion is conclusive, both adrenals were excised, the heart denervated and insulin was administered (chloralose anesthesia). The blood-sugar level declined markedly (121, 56, 14 mg %). The heart rate, before insulin, was 202 beats per minute. As the insulin hypoglycemia developed the heart rate became accelerated, reaching 238 beats per minute.

Since this acceleration occurred in the complete absence of the adrenal glands, we are convinced that the reaction can not be relied upon as an indicator for changes in the rate of epinephrine secretion. In view of these results and those obtained by direct, quantitative measurements of the epinephrine output, we are led to conclude that, regardless of its influence on the blood-sugar, insulin does not detectably alter the rate of liberation of epinephrine from the adrenals.

11193 P

Influence of Ingestion of Pancreatic Juice upon Liver Fat in Depancreatized Dog Maintained with Insulin.*

M. LAURENCE MONTGOMERY, C. ENTENMAN, G. E. GIBBS AND I. L. CHAIKOFF.

From the Division of Surgery (San Francisco) and the Division of Physiology (Berkeley), University of California Medical School.

Previous work has clearly established, first, that completely depancreatized dogs develop fatty livers despite their survival with insulin and a diet adequate in calories, proteins, salts and vitamins, and, second, that raw pancreas, when ingested, possesses not only preventive but curative action upon the deposition of the excessive amounts of lipids in the liver.[1] Moreover, it has been shown that ligation of the pancreatic ducts, a process which completely excludes the pancreatic juice from the intestinal tract and which results in

---

* Aided by grants from the Christine Breon Fund for Medical Research and the Melville Luther Montgomery Donation. Assistance was furnished by the Works Progress Administration (O.P. 65-1-08-62, Unit A6). The insulin used in this investigation was generously furnished by Eli Lilly and Co.

[1] Kaplan, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1937, **119**, 435.

destruction of the acinar elements of the pancreas, is likewise followed by the appearance of fatty livers.[2] These observations would seem to suggest that a factor (or factors) which is capable of controlling the lipid content of the liver resides in the external secretion of the pancreas. It was recognized, however, that proof for this belief is lacking and might be obtained by observing the effects of the ingestion of the *external secretion* of the pancreas upon the liver lipids of the completely depancreatized or duct-ligated dog. The present investigation was undertaken to test this view. It shows that pancreatic juice, when fed over periods of 20 weeks, is quite active in preventing the appearance of excessive deposits of fat in the liver of the completely depancreatized dog maintained with insulin.

The maintenance of depancreatized dogs in this laboratory has been described elsewhere.[1] For a variable period following pancreatectomy each dog received twice daily a diet containing 250 g of lean meat, 50 g of sucrose, 5 g of bone ash and 125 g of raw pancreas from which all visible fat had been removed. Vitamin supplements were furnished twice weekly.† When a vigorous appetite appeared, the feeding of raw pancreas was discontinued and each dog then received pancreatic juice twice daily (Table I) in addition to the meat, sucrose, bone ash and vitamin constituents listed above. The feeding of pancreatic juice was continued for a period of 20 weeks, since it has been shown[1] that this interval is necessary to insure a consistent finding of an excess of 14% fatty acids in the liver of depancreatized dogs when pancreas or other curative agents are withheld.

The pancreatic juice was collected by means of the Elman-McCaughan fistula[3] in dogs that were maintained in good condition with a diet that contained lean meat, raw pancreas, salts and vitamin sup-

TABLE I.
Liver Lipids of Depancreatized Dogs Fed Pancreatic Juice.

Dog	Weight		Period depancreatized, weeks	Pancreatic juice		Liver	
	Initial, kg	Final, kg		Period fed, weeks	Amount daily, avg cc	Wt, g	Fatty acid content, %
D 228	10.5	9.5	28	20	377	530	3.70
D 229	9.9	9.8	28	20	377	540	7.00
D 230	10.1	9.0	28	20	377	570	5.79
D 234	16.7	11.1	24	20	377	535	3.11

[2] Montgomery, M. L., Entenman, C., and Chaikoff, I. L., *J. Biol. Chem.*, 1939, **128**, 387.

† Vitamins A and D were supplied in the form of cod liver oil; the B complex as a rice bran concentrate.

[3] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

plements. Fluid loss was replaced by the daily administrations of 1000 to 1500 cc of Ringer's solution.

At the end of the 20 weeks' period, during which they received pancreatic juice, the depancreatized dogs were sacrificed and the entire liver was removed for lipid analyses. The preparation of the liver for analysis has been recorded elsewhere.[2] The fatty acid content of the livers of 4 dogs is recorded in Table I. It should be noted that these livers contained between 3.1 and 7.0% fatty acid. In 2 of the animals values below 4% were found. These observations are in direct contrast with the results of the previous study, in which it was shown in 30 depancreatized dogs kept alive with insulin, that (although fatty livers may appear as early as 3.5 weeks after excision of the gland) a fatty acid content between 14.4 and 29.2% (average 24%) appeared in the liver when animals were maintained on diets containing no raw pancreas for 19.5-21 weeks.

The observations recorded here show that a factor (or factors) possessing the property of inhibiting the infiltration of excessive deposits of fat in the liver of the completely depancreatized dog maintained with insulin is present in the external secretion of the pancreas. They do not support the contention of Dragstedt, *et al.*,[4, 5] that the factor is unrelated to the external secretion of the pancreas.

---

[4] Van Prohaska, J., Dragstedt, L. R., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 166.

[5] Dragstedt, L. R., van Prohaska, J., and Harms, H. P., *ibid.*, 1936, **117**, 175.

11194

Observations on the Multiple Nature of the "Rat Filtrate Factor."

D. W. Woolley. (Introduced by Kenneth Goodner.)

From the Hospital of the Rockefeller Institute for Medical Research, New York City

The filtrate factor for rats is that material present in fuller's earth filtrates of liver extract which promotes the growth of rats maintained on a basal diet of only pure chemicals. It has been generally assumed that the growth response is due to the presence of one vitamin in the filtrate, although there have been some dissenters from this view.[1, 2] It has recently been shown[3, 4, 5] that relatively pure preparations of the chick antidermatitis factor or pantothenic acid will produce a good growth response in rats in the classical filtrate factor assay. Hence the question arises as to whether pantothenic acid is to be identified with the "rat filtrate factor."

Results of the present investigation show clearly that there are other unknown growth factors for the rat. Pantothenic acid promotes growth in a filtrate-factor-deficient animal only for a relatively short period. It appears that a growth response is obtained only because the young rat has a sufficient store of other growth factors to meet his needs for the relatively short duration of the usual filtrate factor assay. If young male rats are fed a synthetic ration for 4-6 weeks, they will no longer show a growth response when pantothenic acid is subsequently supplied to them. They still respond dramatically when certain liver concentrates in addition to pantothenic acid are added. If the pantothenic acid is added after 3 weeks, rather than after 4-6 weeks, on the synthetic ration, about half of the animals will grow for a week or 2 and then the growth again ceases. Addition of even 200 mg per day of pantothenic acid fails to produce a response, whereas 50 mg will produce good growth in the usual filtrate factor assay. The synthetic ration consisted of sucrose 75, Labco

---

[1] Elvehjem, C. A., Koehn, C. J., and Oleson, J. J., *J. Biol. Chem.*, 1936, **115**, 707.

[2] Mohammed, A., Emerson, O. H., Emerson, G. A., and Evans, H. M., *Science*, 1939, **90**, 377.

[3] Hitchings, G. H., and Subbarow, Y., *J. Nutrition*, 1939, **18**, 268.

[4] Oleson, J. J., Woolley, D. W., and Elvehjem, C. A., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 151.

[5] Lepkovsky, S., private communication.

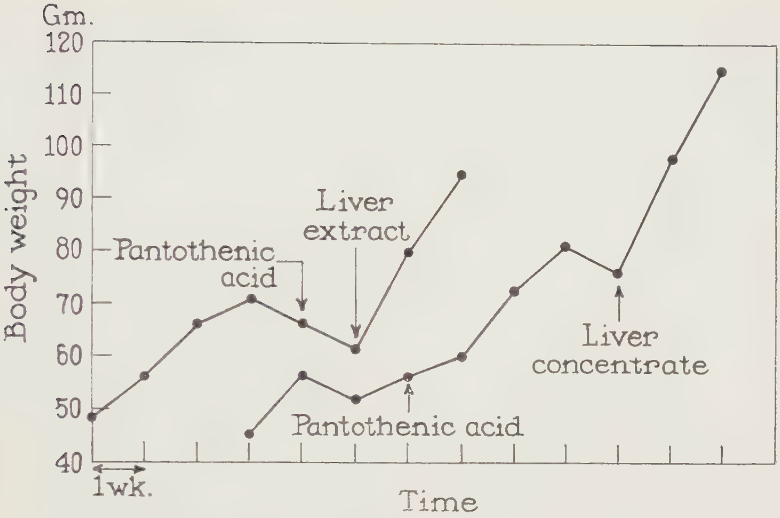


FIG. 1.
Growth responses to pantothenic acid and liver fractions.

casein 18, salts 1[6] 5, cod liver oil 1, corn oil 1, thiamin 200 γ per 100 g, riboflavin 500 γ per 100 g, nicotinic acid 1 mg per 100 g, Merck's synthetic B_6 200 γ per 100 g, β-alanine 1 mg per 100 g, and choline 1 mg per 100 g. Typical growth curves are shown in Fig. 1.

Several sources of the unknown growth factors were compared, and it was found that the fraction of aqueous liver extract which was soluble in 95% alcohol was the richest source. We wish to thank Dr. David Klein of Wilson Laboratories for gifts of this material. One percent of this substance when added to the ration of rats which had failed to grow after the addition of 100 γ per day of pantothenic acid produced a weekly gain of 15 g.

The best method for concentrating the unknown growth factor or factors was found to be as follows: 100 g of the liver extract were extracted thoroughly with butanol. The extracts were treated with an equal volume of acetone, and the filtrate was adsorbed with 100 g of norit A. The active filtrate was concentrated under reduced pressure to a syrup and thoroughly extracted with acetone. The active acetone residue was then dissolved in a small volume of water, made alkaline with barium hydroxide, and treated with 10 volumes of alcohol. The precipitated barium salts were decomposed with H_2SO_4 and were found to contain most of the activity of the original material.

Attempts have been made to show that biotin stimulates the growth of rats. For this work, a highly potent biotin concentrate was made by the method of Woolley, McDaniel and Peterson.[7] When this material was added at a level equivalent in biotin content to 2% of rice bran extract, no growth response was obtained. The same assay technic was used here as with the liver fractions; that is, the rats were fed the synthetic ration for 5 to 6 weeks, then given 100 γ per day of pantothenic acid, and subsequently the biotin concentrate. The ability of the animal to respond was finally proven by addition of one of the potent liver fractions described above. While this does not prove that the rat does not require biotin, it shows that this substance is not the sole remaining growth factor for this species.

---

[6] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

It is not justifiable to ascribe the growth-promoting activity of the above liver concentrate solely to one substance. The experience of the past has emphasized the need for caution in attributing "filtrate factor" activity of concentrates to one compound. However, the above method is a simple and effective means of purifying the remaining unknown rat growth factors. The final answer as to whether only one substance is concerned must await crystallization of the active factor.

11195 P

Acute Circulatory Failure (Shock) Following Subcutaneous Injection of Hypertonic Sodium Chloride Solution.

HARRY A. DAVIS. (Introduced by A. G. Eaton.)

From the Department of Surgery, School of Medicine, Louisiana State University, New Orleans.

Intraärterial injection of hypertonic solutions of NaCl is followed by a marked rise in blood pressure which persists for several hours.[1,2,3] The present study is concerned with the effect of the subcutaneous introduction of hypertonic NaCl solutions upon the circulation of the blood, the purpose being to create an area of excessive osmotic pressure and thus induce a flow of fluid out of the capillaries into the site of injection.

Materials and Methods. Seven dogs, weighing from 5 to 8 kg, were anesthetized with sodium pentobarbital. Solutions of NaCl (25%) were injected subcutaneously into the left hind leg, the dosage being 25 cc per kg of body weight. The blood pressure was recorded in the usual manner after insertion of a cannula into the right carotid artery. Blood obtained from the right femoral vein before and at hourly intervals after the introduction of the solution was studied with respect to hemoglobin content, hematocrit, erythrocytes, non-protein nitrogen, sugar and NaCl.

---

[7] Woolley, D. W., McDaniel, L. E., and Peterson, W. H., *J. Biol. Chem.*, 1939, **131**, 381.

[1] Davis, H. A., Jermstad, R. J., and Choisser, R. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 144.

[2] Davis, H. A., *M. Ann. District of Columbia*, 1937, **6**, 344.

[3] Kendrick, D. B., *Surgery*, 1939, **6**, 520.

Results. All the animals died within 5 to 10 hours. Immediately after the injection the systolic and diastolic blood pressures rose 10-15 mm Hg above the initial level, then fell to the original level, at which they were maintained for 1-3 hours. During this period the blood showed an increasing concentration. The heart rate was accelerated and the respirations became rapid and shallow. The blood pressure fell slowly to the shock level (80 mm Hg) and thereafter fell relatively rapidly. The mucous membranes of the mouth and tongue showed an increasing cyanosis, and the blood obtained by venipuncture was dark and appeared poorly oxygenated. The amount of urine formed during each experiment varied from 20 to 100 cc.

Edema developed at the site of injection and frequently extended to the abdominal wall and even to the outer aspect of the left thorax. Its extent seemed to bear a definite relation to the survival time and was greatest when the period of survival was longest.

The erythrocyte counts showed increases varying from 20 to 63%, the hematocrit reading rose 37 to 47% above the initial level, and the amount of hemoglobin was increased by 23 to 73%. These figures suggest that a considerable diminution of the plasma volume had occurred. The blood sugar was increased by 108 to 151%, the blood non-protein nitrogen by 76 to 137%, and the whole blood chloride (estimated as NaCl) by 31 to 50%. Representative data are shown in Table I.

The significant rise in the blood chloride level must be commented upon. In other forms of shock a fall takes place, and the rise in these experiments may be attributed to absorption of NaCl from the site of injection into the blood stream. A simple calculation will reveal that a total of only 2-3 g of NaCl was absorbed. The intravenous administration of similar or larger amounts in the form of hypertonic solution results in a definite hemodilution, which is in contradistinction to the hemoconcentration found in the present experiments. By altering the stated dosage of hypertonic NaCl solution it was possible to hasten or delay the time of onset of shock.

TABLE I.

Dog wt, kg	Blood pressure, mm Hg		Hematocrit, %		R.B.C., millions per cm		Hb, %		Blood sugar, mg %		Blood non-protein N, mg %		Blood NaCl, mg %	
	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.	B.I.	A.I.
6.8	165	10	38	56	5.5	8.98	68	118	72	181	34.2	60	650	890
6.4	135	35	48	57	7.94	9.22	98	115	89	136	19.8	30	678	992
8.0	160	5	42	58	6.94	10.6	85	150	84	52	42.6	60	736	1110

B.I. = Before injection of hypertonic NaCl solution.
A.I. = After injection of hypertonic NaCl solution.

Conclusions. The subcutaneous injection of hypertonic NaCl solution is followed by acute circulatory failure, which is associated with hemoconcentration and with an increase in the amount of sugar, non-protein nitrogen, and NaCl in the blood. Other hypertonic solutions, such as hypertonic glucose solution, when given subcutaneously, might be expected to produce somewhat similar results.

11196 P

Relative Significance of Water and of Protein Loss in Dehydration Shock.

HARRY A. DAVIS. (Introduced by A. G. Eaton.)

From the Department of Surgery, School of Medicine, Louisiana State University, New Orleans.

The importance of loss of fluid at the site of injury has been emphasized in shock due to trauma or burns and associated with hemoconcentration.[1, 2] The chemical composition of this fluid is similar to that of blood plasma and its total protein is approximately 5.2 g per 100 cc.[3] The present study was undertaken to determine the relative significance of loss of the protein fraction and of the aqueous fraction of the blood plasma in the production of acute circulatory failure associated with hemoconcentration.

Materials and Methods. Shock was induced in 6 dogs which had been anesthetized with sodium pentobarbital, by the subcutaneous introduction into the left leg of 25% sodium chloride solution, the dosage being 25 cc per kg of body weight. Details of this method have been published elsewhere.[4] Determinations of the hemoglobin, hematocrit and erythrocytes were made upon blood withdrawn from the right femoral vein before and during the course of each experiment. After death each animal was bisected and the difference in weight between the halves of the body was determined. In estimating the amount of fluid lost at the site of injection, a correction was made by subtracting from the total weight the weight of the injected solution; 100 cc of 25% solution was found to weigh approximately 118.8 g, and the calculations were made upon this basis.

---

[1] Blalock, A., *Arch. Surg.*, 1930, **20**, 959.

[2] Parsons, E., and Phemister, D. B., *Surg., Gynec. and Obst.*, 1930, **51**, 196.

[3] Harkins, H. N., and Harmon, P. H., *Ann. Surg.*, 1937, **106**, 1070.

[4] Davis, H. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 354.

TABLE I.

Dog wt, kg	Amt injection 25% NaCl sol., cc	Calc. plasma vol., cc	Reduction plasma vol. (calc. from hematocrit), cc	Corrected local fluid loss, cc	Local fluid loss, % body wt	Total protein in fluid, g %	Estimated total protein loss at site of injection in g
6.2	155	319	141	263	4.2	0.4	1.052
6.4	160	275.6	123	260	4.0	1.0	2.6
6.6	165	410.7	242	602	9.1	1.2	7.224

The fluid obtained at the site of injection was filtered and analyzed quantitatively for total protein.

Results. After correction, the amount of fluid lost at the site of injection varied from 4 to 9.1% of the body weights of the animals. The average loss of fluid was 5.9% of the body weight.

It is now necessary to consider to what extent these figures represent a loss of fluid from the blood stream. It is evident, from representative data shown in Table I, that the calculated reduction in the fluid portion of the blood is less than the amounts of fluid lost at the site of injection, these figures being based upon alterations of hematocrit, erythrocyte and hemoglobin readings. This would suggest that part of the fluid is lost from the interstitial tissue spaces.

The total protein content of the fluid lost at the site of injection varied from 0.4 to 1.7 g per 100 cc. The low protein content appears to indicate that the hemoconcentration is due not to a loss of whole plasma but rather to a loss of the aqueous fraction. One cannot exclude the possibility of slight damage to the capillary walls at the site of injection. Such an injury would permit the escape of whole plasma, but if it is present, the low protein content of the fluid suggests that it is apparently not significant. It has been shown that death will occur in dogs if whole plasma to the extent of 2.6 to 4.4% of the body weight is removed,[5, 6, 7] which indicates that animals will tolerate the loss of large amounts of plasma protein before death takes place. In these experiments, however, 2 animals died (Table I) with local fluid losses amounting to only 4 and 4.2% of the body weight respectively. In both instances the fluid was almost entirely water and its protein content was very low.

Conclusions. Shock which follows the subcutaneous injection of hypertonic NaCl solution can be explained by a loss of fluid at the site of injection. This fluid contains only small amounts of protein, and it is suggested that it consists partly of water derived from the blood and partly of water derived from the interstitial tissue spaces. The presence of hemoconcentration is due to a loss of the aqueous fraction of the plasma rather than to a loss of whole plasma. Rapid loss of water from the blood is capable of producing acute circulatory failure.

[5] Johnson, G. S., and Blalock, A., *Arch. Surg.*, 1931, **22**, 626.

[6] Harkins, H. N., and Harmon, P. H., *Surgery*, 1937, **1**, 276.

[7] Roome, N. W., Keith, W. S., and Phemister, D. B., *Surg., Gynec. and Obst.*, 1933, **56**, 161.

11197 P

Liberation of Histamine by Trypsin.

M. Ramirez de Arellano, A. H. Lawton and C. A. Dragstedt.
From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Rocha e Silva[1] has reported that trypsin has the capacity of liberating histamine when perfused through the guinea pig lungs and postulates that the toxicity of trypsin when injected intravenously in intact animals may be due to this effect. The present report is concerned with confirming his findings on the isolated perfused guinea pig lung and of reporting observations on the liberation of histamine in intact dogs following its intravenous administration.

The isolated lungs of guinea pigs were perfused through the pulmonary artery with Sollmann-Rademaeker's solution according to the method of Feldberg and Kellaway.[2] They were rhythmically ventilated with air throughout the experiment. After a control perfusion of about 30 minutes a small amount of trypsin was injected into the afferent cannula and the perfusate then tested for histamine activity. Two highly active, but not crystalline, preparations of trypsin were used in amounts from 0.5 to 10 mg. In the experiments done to date the smaller dosage occasionally led to the appearance of histamine activity in the perfusate, while doses of more than 1.0 mg did so regularly. Since this experiment is essentially confirmatory of the findings of Rocha e Silva, further details are omitted.

Four dogs were anesthetized with ether and barbital, and arranged for the recording of blood pressure tracings. Normal blood samples were obtained and then trypsin was injected intravenously in doses of 2 to 5 mg per kilo. Blood samples were obtained 2 to 5 minutes after the injection and all samples of blood then assayed for their histamine content by the method of Barsoum and Gaddum[3] as modified by Code.[4] In one experiment there was but a slight increase in the blood histamine, in 2 experiments there was a moderate increase and in one experiment there was a very marked increase from a normal value of no detectable histamine to a level of 1.8 γ of histamine base per cc after the trypsin injection. There was a marked fall of blood pressure in each instance, being only temporary in the first 3 instances, while continuing until death of the animal in the last instance. In 2 other animals a portion of the liver was excised (using an electro-surgical knife to obviate bleeding*) before and after the trypsin injection. The liver samples were assayed for their histamine content by the method of Best and McHenry. In one experiment the liver content of histamine decreased from a normal value of 66 γ histamine base per gram to a value of 33 indicating a liberation of 10 mg for the whole liver, and in the other experiment there was a decrease from 50 γ per gram to 17 indicating a liberation of 6 mg from the entire liver.

---

[1] Rocha e Silva, *Arquivos d. Inst. Biol.*, 1939, **10**, 93.

[2] Feldberg, W., and Kellaway, C. H., *J. Physiol.*, 1937, **90**, 257.

[3] Barsoum, S. S., and Gaddum, J. H., *J. Physiol.*, 1936, **85**, p. 2.

[4] Code, C. F., *J. Physiol.*, 1937, **89**, 257.

It is thus clear that trypsin, like proteoses and certain snake venoms has the ability to liberate histamine from the tissues of the intact animal. Enough experiments have not as yet been done to determine whether the toxicity of trypsin can be exclusively related to this property.

11198

Possible Physiologic Significance of the Zinc Content of Insulin.

O. Hechter, R. Levine and Samuel Soskin.

From the Department of Metabolism and Endocrinology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.*

It is known that the effectiveness of insulin in lowering the blood sugar level is dependent upon the integrity of the S-S groups which form part of its protein structure.[1] This suggested the possibility that insulin might exert its physiologic action through the influence of its S-S groups upon such tissue enzymes as are dependent upon S-H groups for their activity.[2-5] We therefore studied the *in vitro* effect of insulin upon succino-oxidase activity. A significant effect was found. But. it soon became apparent that this *in vitro* action

---

* We are indebted to Dr. Robert S. Hardwick for the surgical operations in these experiments.

* Aided by the Max Pam Fund for Metabolic Research.

[1] Jensen, H. F., *Insulin*, Oxford University Press, London, 1938.

[2] Hopkins, F. G., Morgan, E. J., and Lutwak-Mann, C., *Biochem. J.*, 1938, **32**, 1829.

[3] Rapkine, L., *Biochem. J.*, 1938, **32**, 1729.

[4] Hellerman, L., *Physiol. Rev.*, 1937, **17**, 454.

[5] Purr, A., *Biochem. J.*, 1935, **29**, 5.

TABLE I.
Effect of Crystalline Insulin on Succino-Oxidase Activity *in vitro.*

Amount of insulin added per vessel	μl O_2 uptake in 20 min	40 min	60 min	% inhibition in 60 min
0	131	218	276	—
0.27 mg (6 units)	99	182	220	20.3
0.54 mg (12 units)	77	124	165	40.0

All vessels contained: 1 ml enzyme solution, 0.4 ml M/10 succinate and M/15 phosphate buffer (pH 7.4) to make a total volume of 3 ml. Gas phase; air.

of insulin did not depend upon its S-S groups, but rather upon its zinc content.

Methods and Results. An active succino-oxidase preparation was obtained from minced, fresh beef heart. Its activity was determined by measuring the oxygen consumption in the presence of excess succinate in the Warburg apparatus (38°C; gas phase—air; pH 7.4).

Table I presents a typical experiment of a large number which consistently showed a significant inhibition of the succino-oxidase system by crystalline insulin.†

To test whether this action of insulin ran parallel with its hypoglycemic effects *in vivo,* experiments were repeated using a sample of crystalline insulin which had been previously inactivated as regards its *in vivo* effects by boiling in dilute alkaline solution. Table II shows that this "inactivated" insulin still retained its full inhibitory action on the succino-oxidase system.

In view of these results further experiments were performed to see whether the *in vitro* activity of insulin depended upon its content of zinc. Accordingly we compared the inhibitory effects of our crystalline insulin (zinc content 0.47%) with that of amorphous insulin† of equal *in vivo* potency (zinc content 0.18%). It may be seen from Table II that the amorphous insulin containing less zinc, exerted no significant inhibitory influence. Furthermore, zinc sul-

TABLE II.
Effect of Zinc, in Insulin and Alone, on Succino-Oxidase Activity *in vitro.*

Additions	μl O_2 uptake in 60 min	% inhibition
0	258	—
0.54 mg crystalline insulin (2.5 γ Zn)	200	24
0.54 mg crystalline insulin "inactivated"	188	27
0.54 mg amorphous insulin (0.97 γ Zn)	255	1
Zinc sulphate solution containiing 2.5 γ Zn	180	30

Contents of vessels before additions as in Table I.

---

† We are indebted to Dr. Rhodehamel and the Eli Lilly Company, for supplies of pure crystalline and amorphous insulin.

phate in a concentration equivalent to the zinc content of the amount of crystalline insulin which we employed (*circa* 1×10^{-5} M) caused a similar inhibition in the absence of insulin.

Discussion. The *in vitro* inhibition of succino-oxidase activity by insulin is due solely to the zinc content of the insulin sample, and can be duplicated by equivalent amounts of the zinc ion. The possible physiologic significance of these results depends upon whether zinc is an integral part of the insulin molecule as secreted by the pancreas and, if so, whether the zinc moiety exerts a similar effect *in vivo*. The work of Scott and Fischer[6] and of Cohn, *et al.*,[7] indicates that zinc is very closely associated with the insulin molecule. Crystalline insulin has a constant percentage content of zinc, which is not decreased by repeated recrystallizations, and which cannot be separated from the protein by electrodialysis. However, even if it may be assumed, for the time being, that zinc is an integral part of the insulin molecule as secreted, it still remains to be shown that it is effective *in vivo*. Recent work has shown that a number of other enzyme systems may be influenced by very small concentrations of zinc *in vitro*.[8, 9] But evidence is lacking as regards similar *in vivo* actions of zinc, either when associated with insulin or by itself.

11199

Effect of NaCl and Desoxycorticosterone on Body Weight and Carbohydrate Stores of Adrenalectomized Rats.*

EVELYN ANDERSON AND VIRGIL V. HERRING. (Introduced by H. M. Evans.)

From the Institute of Experimental Biology and the Department of Medicine of the University of California, Berkeley and San Francisco, California.

It is well known that additional amounts of sodium chloride will enable adrenalectomized animals to survive a considerable length of time beyond the expected survival period following adrenalectomy. It has been found that adrenalectomized rats given 1% solution of

---

[6] Scott, D. A., and Fischer, A. M., *Biochem. J.*, 1935, **29**, 1048.

[7] Cohn, E. J., Ferry, J. D., Lingood, J. J., and Blanchard, M. H., *Science*, 1939, **90**, 183.

[8] Thunberg, T., *Skand. Arch. Physiol.*, 1934, **69**, 247.

[9] Lohmann, K., and Kossel, A. J., *Naturwis.*, 1939, **27**, 595.

* We wish to acknowledge the assistance of the Federal Works Progress Administration, Project No. OP 65-1-08-62, Unit A-5, and the Christine Breon Fund.

NaCl in place of tap water to drink *ad libitum,* will excrete sodium and potassium as normal animals in contrast to adrenalectomized rats unsupported by salt which show an increased urinary excretion of sodium and a retention of potassium.[1] It has also been shown that sodium chloride therapy enables adrenalectomized animals to absorb glucose from the intestinal tract like normal animals, in contrast to untreated adrenalectomized animals which have a decreased intestinal absorption of glucose.[2]

This study was undertaken in order to compare the general nutritional status and the carbohydrate levels of the body after glucose feeding in adrenalectomized rats treated with sodium chloride, with that of adrenalectomized rats receiving no treatment, adrenalectomized rats treated with desoxycorticosterone and rats in which the adrenals were intact. Male rats 63 days of age were used. The animals were kept in individual cages in a constant temperature chamber at 28°C for 2 weeks before operation and during the 10-day observation period following operation. A record of the daily intake of food and fluid and of body weight was kept during the entire 24-day period. A summary of these data is given in Table I.

In Group I, consisting of 10 rats, the adrenals were removed and the animals were started immediately on a 1% solution of NaCl to drink *ad libitum.* In Group II, consisting of 10 rats, the same operation was performed and the animals put on a daily dose of 0.5 mg desoxycorticosterone, administered in oil by the subcutaneous route. They received tap water to drink. In Group III, consisting of 8 rats, a "mock adrenalectomy" was performed, that is, the adrenals were dissected free from the surrounding tissue, but were not removed. These animals received no therapy and were given tap water to drink. In Group IV, consisting of 5 rats, the adrenals were removed completely and no therapy given. This group was sacrificed on the 4th day post-adrenalectomy, in contrast to the animals of the other 3 groups which were not sacrificed until the 10th day. During the 10-day post-operative period the NaCl-treated animals each received a daily average of 38 cc of a 1% NaCl solution. In Table I it is shown that the average daily food intake and the increase in body weight were practically the same for the first 3 groups of animals. The corresponding figures for Group IV might well be considered comparable for the shorter period.

The day before sacrificing, the rats were started on a 20-hour fast.

---

[1] Anderson, E., and Joseph, M., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 347.

[2] Althausen, T. L., Anderson, E., and Stockholm, M., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 342.

TABLE I.
Intake of Food and Fluid and Changes in Body Weight in Treated Adrenalectomized Rats.

	No. animals	Avg body wt (g)		Avg daily food intake (g)		Avg daily fluid intake (cc)	
		At operation	10th day post op.	Before operation	After operation	Before operation	After operation
Group I							
Adrenalectomized. 1% NaCl	10	194	218	14.9	10.8	29 H_2O	38 1% NaCl
Group II							
Adrenalectomized. 0.5 mg desoxycorticosterone	10	189	212	15.4	10.5	26 H_2O	20 H_2O
Group III (controls)							
"Mock adrenalectomized"	6	191	217	14.4	12.3	26 H_2O	26 H_2O
Group IV (controls)							
Adrenalectomized, untreated	5	172	180*	15.6	14.1	—	—

* 4th day postoperative

TABLE II.
Carbohydrate Stores After Adrenalectomy in Rats Treated with 1% NaCl, and with Desoxycorticosterone.
Average.

	No. animals	Body wt, g (20 hr fasting)	Glucose absorbed per 100 g B. wt per hr	Blood sugar, mg %	Liver glycogen		Muscle glycogen mg % of wet tissue
					mg %§	mg per 100 g body wt	
Group I							
Rats adrenalectomized for 10 days, given 1% NaCl	10	207 (180-234)	136 (116-149)	133 (90-305)	1,494 (823-2,271)	47.5 (25.8-78.9)	606* (504-757)
Group II							
Rats adrenalectomized 10 days, given 0.5 mg desoxycorticosterone daily	10	200 (175-226)	142 (112-157)	119 (90-190)	1,730 (999-2,485)	49.9 (30.8-67.8)	630† (485-757)
Group III (control)							
Rats "mock adrenalectomized" 10 days previously	6	200 (188-216)	141 (133-157)	138 (110-160)	1,760 (1,279-2,251)	60 (36.6-81.1)	712‡ (678-777)
Group IV (control)							
Adrenalectomized 4 days, no treatment	5	176 (163-190)	—	173 (130-310)	292 (86-516)	11 (3.3-22.4)	529 (480-569)

*Values for 7 rats. † Values for 8 rats ‡ Values for 5 rats §Based on wet tissue weight.

The sodium chloride-treated animals continued to have access to the NaCl solution, the rest of the animals were given tap water to drink. Four hours before sacrificing, the animals were given 1.2 g glucose (5 cc of a 25% solution) by stomach tube. The animals were anesthetized with sodium amytal, the tissues removed immediately and the following determinations made: intestinal absorption of glucose; blood sugar; liver glycogen and muscle glycogen. The results of this study are shown in Table II. It will be noted that the values for the glucose absorption and the carbohydrate levels in the blood, liver and muscle are very much the same in the first 3 groups which shows that the salt-treated adrenalectomized rats are able to absorb and store fed glucose as well as the adrenalectomized rats which were treated with a half milligram dose of desoxycorticosterone daily, and as the rats with their adrenals intact. On the other hand the untreated adrenalectomized rats of Group IV showed liver glycogen stores greatly reduced as early as the 4th day post-operative.

Summary. Adrenalectomized rats given a 1% solution of sodium chloride to drink *ad libitum* in place of tap water for a 10-day period after operation gain in weight and are able to store fed glucose in amounts comparable to that of adrenalectomized rats given 0.5 mg desoxycorticosterone daily or to that of rats in which the adrenals are intact.

11200

A Modified Hemmingsen-Krarup Mammalian Activity Recorder.*

ORLANDO PARK AND L. P. WOODS. (Introduced by C. D. Turner.)

From the Department of Zoology, Northwestern University.

Studies in mammalian activity have made necessary the development of recording equipment.[1] Most of these recorders have been simply constructed, utilizing rubber bands, springs or excentric drives. The Hemmingsen-Krarup recorder[2] is an apparatus of considerable precision and a modified form of this equipment has been developed for the study of periodic activity of small mammals.

---

* Recording apparatus constructed by C. P. Clare & Co., Chicago, by means of a grant from Northwestern University.

[1] Park, O., *Ecological Monographs*, 1940, **10** (July).

[2] Hemmingsen, A. M., and Krarup, N. B., *Biologiske Meddel.*, 1937, **13**, 1.

This recorder is depicted in the photographs (Figs. 1, 2) and may be described as follows. The machine consists of 3 main parts: (1) An upright plywood partition (35″ x 34″) for support of the revolving cage, living cage, and mechanism for gearing down and recording activity. (2) The revolving cage (26″ diameter x 8″ deep) constructed of quarter-inch mesh wire on circular plywood back. (3) Living cage (23″ x 6″ x 14″) hinged to the vertical partition at one end and suspended by a spring, attached to the kymograph bracket at the other end. Living cage and revolving cage are in communication by an opening (4″ diameter) cut through the vertical partition.

The gearing mechanism noted above may be described as follows: The revolving cage turns on a ball-bearing racing bicycle axle which has a double excentric on one end. One of these excentrics is connected with a notched sprocket arm which turns a cyclometer fastened on a vertical partition. The second excentric works upon a reduction gear, the periphery of which has 250 teeth. This gearing wheel in turn carries an excentrically attached, adjustable rod and

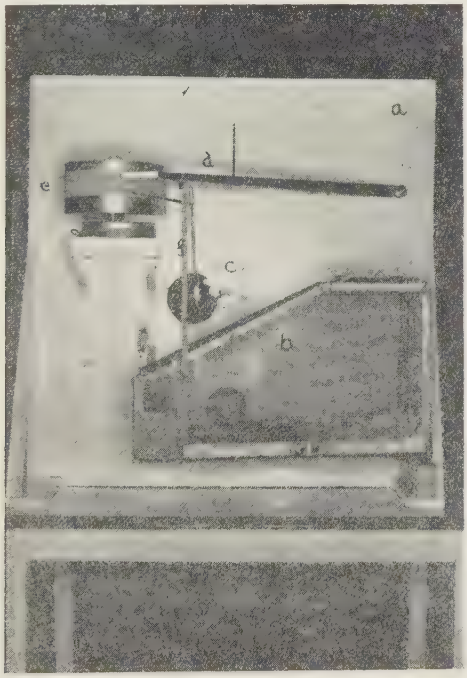


FIG. 1.

Activity recorder: front view. a. Vertical partition; b. Living cage; c. Reduction gear, sprocket arm and cyclometer assembly; d. Recording lever; e. aluminum drum and clockworks; f. Auxiliary lever recording activity in living cage.

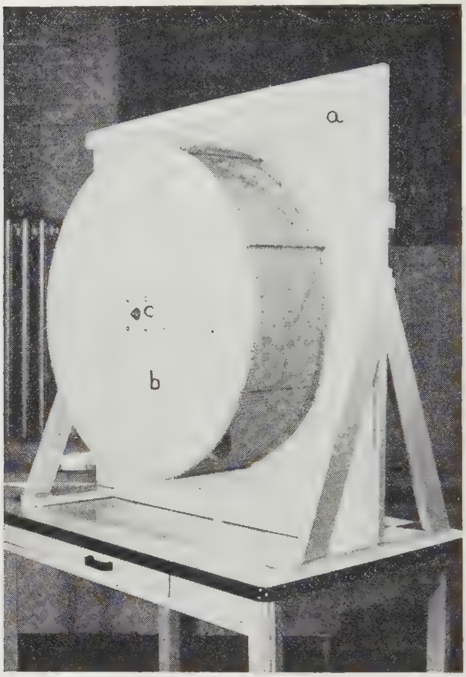


FIG. 2.

Activity recorder: rear view. a. Vertical partition; b. Revolving cage, note circular aperture passing through upright partition and giving access from living cage (Fig. 1) to revolving cage; c. Hub of racing bicycle axle passing through partition to cyclometer assembly.

this rod moves the recording lever in a vertical plane. The recording lever completes a single vertical movement for every 125 revolutions of the revolving cage, or a single complete peak for every 250 revolutions. This worked out at 34 feet traveled by the revolving cage for every millimeter of vertical movement by the recording stylus.

The activity record is made by a spring steel point on the recording lever, writing upon a waxed-paper record. This waxed paper gives a perfect account of the revolving cage, and is carried on an aluminum drum (8″ diameter) revolving once in 24 hours through the agency of a Taylor clockworks. Any activity in the living cage is also recorded on the waxed-paper record. This is accomplished by means of an auxiliary, adjustable recording stylus attached to the free-swinging, spring-suspended end of the living cage. Consequently, activity of the experimental animal is recorded either while moving in the revolving cage, or while eating, drinking, *et cetera,* in the living cage. The axle of the revolving cage is covered by a sheet iron gable guard which prevents the animal from sitting on the axle and rotating the revolving cage.

This recorder was planned in the autumn of 1938, and was ready for service in February, 1939, and has proven accurate and reliable. The following sample brings out the diurnality of a small mammal, held under controlled conditions for 44 days and nights. The chipmunk (*Tamias striatus griseus* Mearns) was used. The recorder was placed in an insulated cabinet, in which the air temperature varied between 71°-73°F for the length of the experiment. Air in the cabinet was slowly changed by a light-tight ventilating pipe attached to an electric fan. Relative humidity varied between 64%-68%, and the recorder was in total darkness throughout the experiment save for a 10-minute interval each 24 hours at which time the waxed-paper record was changed and food and water supply replenished. No adjustments of the apparatus were necessary and the living cage was not cleaned during the experiment so that the animal was not disturbed.

Under these conditions of constant darkness, temperature and humidity the activity was analyzed, and the diurnality is demon-

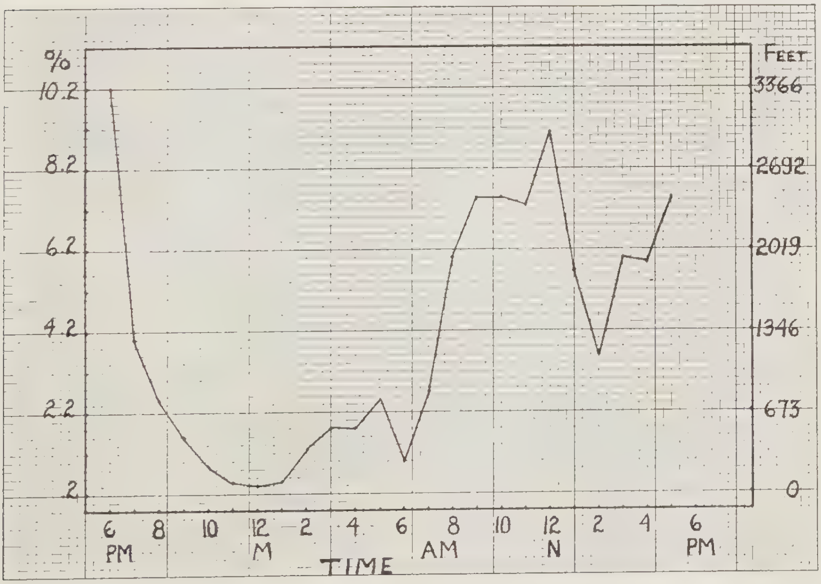


FIG. 3.

Average hourly activity of the chipmunk *(Tamias griseus)* for 44 days and nights in approximately constant temperature, relative humidity and total darkness. On left read hourly average expressed as the percentage of the total 24-hour average activity (approximately 18% of total experimental activity in revolving cage between 7 p.m. and 6 a.m. and 82% between 7 a.m. and 6 p.m.). On right read average number of feet traveled in the revolving cage.

strated in the average activity graph (Fig. 3) of a single female chipmunk from May 17 to June 29, 1939. Complete analysis of the activity of the species cannot be given here, but the activity curve shows that the species is diurnal with activity largely during the period between 6 a. m.-noon, and 2-6 p. m. This coincides with field observation. Analysis of activity by weeks shows that total average activity tends to increase and the periodicity to break down through the experimental time, thus average activity for the first week was much less, and confined to the day, no activity being recorded between 8 p. m.-6 a.m. During the course of this sample experiment the chipmunk traveled an average of 6.2 miles per 24-hour period, or a total of 272.8 miles. The cyclometer reading at this time was 272.6 miles, a difference of .2 miles between the direct count on the revolving axle, and the waxed-paper record, attesting to the accuracy of the apparatus. Such a recorder should be valuable for analysis of small mammal activity cycles in general.

Summary. A modification of the Hemmingsen-Krarup activity recorder is described and figured. In a test of the apparatus, the chipmunk was found to have at least an habitual diurnality in constant darkness, temperature and relative humidity.

11201

Effects of Adrenalectomy on the Blood Count of the Rat.

A. J. DALTON AND G. MASSON.

From the Department of Anatomy, McGill University, Montreal, Canada.

Study of the literature concerning changes in the blood picture during adrenal insufficiency reveals that the results reported by different workers are not in complete agreement. While the experiments of Corey and Britton[1] show an increase in the red cells and lymphocytes, accompanied by a decrease in the neutrophiles, those of Simpson, Dennison and Korenchevsky[2] show a decrease in the red cells and lymphocytes with not much change in the total white cell count, whereas the effects observed by Shecket, Friedman and Nice[3] were leukocytosis, lymphocytosis and neutropenia.

---

[1] Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1932, **102**, 699.

[2] Simpson, S. L., Dennison, M., and Korenchevsky, V., *J. Path. and Bact.*, 1934, **39**, 569.

[3] Shecket, H. A., Friedman, D. L., and Nice, L. B., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 608.

TABLE I.

Rats	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
R.B.C.	5,000,000 11,950,000	6,500,000 9,800,000	6,000,000 10,650,000	9,950,000 11,800,000	8,750,000 10,350,000	8,720,000 11,520,000	7,680,000 8,920,000	8,160,000 11,640,000
L.L.	1,500 6,000	500 7,500	1,500 4,000	1,500 4,000	1,000 4,500			
S.L.	11,500 21,000	11,000 17,000	13,000 19,000	26,500 52,500	11,500 22,000	9,000 11,000	11,500 14,000	9,000 11,500
Poly.	4,500 13,500	4,000 13,000	1,000 6,500	5,000 18,500	4,500 9,000	3,500 14,000	500 8,500	1,500 30,500
Total White cells	17,500 40,500	15,500 37,500	15,500 29,500	33,000 75,000	17,000 35,500	12,500 25,000	12,000 22,500	10,500 42,000

The first value given for each category of cell represents the count before operation, and the second, the count 24 hours before death.

The difference in these results led us to repeat these experiments.

In a first experiment, six male hooded rats, weighing 150-200 g, were adrenalectomized and blood taken from the tail was examined at regular intervals until their death. The results obtained for each category of blood cell did not permit us to draw definite conclusions; for example, 3 of the rats showed a decrease in the red cells, the others, an increase. We believed that these results, similar to those reported before by other workers, were caused by differences in the ability of individual animals to resist surgical shock and hemorrhage subsequent to the operation, as well as to small variations in the degree of shock and hemorrhage produced.

In a second experiment, therefore, 8 male rats of similar weights were given 1 cc injections of Wilson's cortical extract daily for 3 days following adrenalectomy. Thus we eliminated any individual differences due to operative procedure.

Two days after cessation of the cortin injections, the animals began to show symptoms of adrenal insufficiency, and from this time on, their blood was examined daily until their death.

The results obtained, all uniform, were as follows: the red blood cell count showed a rapid increase during the first 2 or 3 days after discontinuation of cortin treatment, and remained well above normal until death. The large lymphocytes, the small lymphocytes, and the neutrophiles, after small irregular variations, increased markedly in number during the 24-hour period preceding death.

Reticulocyte counts made on the blood of these rats agree with our previous finding[4] that after adrenalectomy a great increase in reticulocytes occurs, followed by a drop to nearly normal numbers before death.

---

[4] Dalton, A. J., Masson, G., and Selye, H., *Bull. et Mém., Soc. Endocrine Roumanie,* 1939, **5**, No. 6.

11202 P

Evidence for the Detoxication of Diphenyl Through a Sulfur Mechanism.

HAROLD D. WEST. (Introduced by E. L. Turner.)

From the Department of Biochemistry, Meharry Medical College, Nashville, Tennessee.

Naphthalene[1, 2] and anthracene[3] as well as monohalogenated benzenes[4, 5] fed to animals are converted in part to mercapturic acids. Increased neutral sulfur excretion following the administration of benzene[6, 7] and phenanthrene[7] seems to indicate that these hydrocarbons are similarly detoxicated. Growth of rats fed a casein-low ration is inhibited by incorporation in the diet of bromobenzene,[5] naphthalene,[8] phenanthrene,[9] certain complex hydrocarbons—both carcinogenic and non-carcinogenic,[10] or other substances.[11, 12] This deficiency may be relieved by simultaneous administration of dl-methionine or of l-cystine and certain of its derivatives. Presumably hydrocarbons produce a deficiency in the sulfur-containing amino acids available to the organism, because of the preferential utilization of these acids for detoxication reactions. Thus added evidence is available for mercapturic acid formation by phenanthrene and certain of the more complex hydrocarbons.

The chemical relationship of diphenyl to bromobenzene, and to naphthalene, anthracene, and other aromatic hydrocarbons previously studied suggests the possibility that this substance may be handled similarly by the animal organism. Preliminary results from growth experiments obtained in a study of the fate of this hydrocarbon are now being reported.

---

[1] Bourne, M. C., and Young, L., *Chem. and Ind.*, 1933, **52**, 1056; *Biochem. J.*, 1934, **28**, 803.

[2] Stekol, J. A., *J. Biol. Chem.*, 1936, **113**, 675; 1935, **110**, 463

[3] Boyland, E., and Levi, A. A., *Biochem. J.*, 1936, **30**, 1225.

[4] Lawrie, N. R., *Biochem. J.*, 1931, **25**, 1037.

[5] White, A., and Jackson, R. W., *J. Biol. Chem.*, 1935, **111**, 507.

[6] Callow, E. H., and Hele, T. S., *Biochem. J.*, 1926, **20**, 598.

[7] Stekol, J. A., PROC. SOC. EXP. BIOL. AND MED., 1935-36, **33**, 170.

[8] Stekol, J. A., *J. Biol. Chem.*, 1937, **121**, 87.

[9] Stekol, J. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 328.

[10] White, J., and White, A., *J. Biol. Chem.*, 1939, **131**, 149.

[11] White, A., *J. Biol. Chem.*, 1935-36, **112**, 503.

[12] Simon, E. E., and White, A., Proc. Am. Soc. Biol. Chem., *J. Biol. Chem.*, 1938, **123**, p. cix.

Albino rats were used in the experiments. The basal diet (Diet 1-A) was similar to that used by White and Jackson[5] except that dextrin and a small amount of agar agar were used in place of the starch. It contained casein, 6%; salt mixture,[13] 4; sucrose, 15; dextrin, 48; agar agar, 2; lard, 22; and cod liver oil, 3. When a diet was supplemented, the dextrin was reduced by the amount of the supplement. Diet 1-D contained 1% of diphenyl. Diets 1-D-C, 1-D-M, 1-D-T, and 1-D-S contained, in addition to 1% of diphenyl, 0.120% of l-cystine, 0.150% of dl-methionine, 0.250% of taurine and 0.750% of sodium sulfate (anhydrous) respectively. Vitamin B factors were furnished in the form of pills given once daily to each rat. Each pill contained 0.4 g of brewers' yeast powder and a small amount of dextrin. Water and food were given *ad libitum.*

Results of the experiments are given in Table I. In each case complete failure of growth invariably ensued immediately when a diet was supplemented either with diphenyl alone or together with

TABLE I.*

Inhibition of growth of young rats† by diphenyl on diets low in casein and the subsequent effects of supplementation with l-cystine, dl-methionine, taurine, or sodium sulfate.

Sex	Rat No.	Days	Diet	Total change in wt	Daily food consumption	Wt at beginning of each period
♂	11	36	1-A	18.2	4.7	67.1
		32	1-D	—11.9	3.9	85.3
♂	8	16	1-A	15.4	5.5	64.5
		24	1-D	—12.0	3.4	79.9
		32	1-D-C	9.2	5.0	67.9
♂	10	16	1-A	7.3	4.1	60.1
		24	1-D	— 7.5	3.5	67.4
		32	1-D-M	6.2	3.8	59.9
♀	14	16	1-A	4.1	4.4	63.5
		44	1-D	— 6.5	3.1	67.6
		8	1-D-T	— 2.3	3.3	61.1
♀	15	16	1-A	6.3	5.8	68.7
		44	1-D	— 8.8	3.7	75.0
		8	1-D-S	— 0.4	4.0	66.2

* The data for rat 11 is typical of results with 3 animals; for rat 8, 4 animals; for rat 10, 5 animals; for rat 14, 3 animals; and for rat 15, 3 animals.

† Histological examination of livers and kidneys of 2 animals which had received Diet 1-D for several weeks was carried out by Dr. J. R. Cuff of the Department of Pathology, who is due our thanks. Beginning fatty change was observed in the livers and mild toxic (tubular) change was seen in the kidney sections.

---

[13] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, **37**, 572.

taurine or sodium sulfate. When diets containing diphenyl were supplemented with l-cystine or dl-methionine, however, the deficiency imposed by the diphenyl was promptly removed and growth was resumed. The results indicate that diphenyl is handled in the organism of the rat by some mechanism which involves the sulfur of sulfur-containing amino acids ordinarily available for growth.

11203

Prolonged Maintenance of a Mammary Carcinoma in Vitamin E-Deficient Rats.*

W. LAMAR BRYAN AND KARL E. MASON.
From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tenn.

Observations concerning a possible relationship between vitamin E and the incidence and growth of neoplasms are fragmentary, and are characterized by wide differences in the type of animal, neoplasm, and experimental method used. Adamstone[1] has described the occurrence of a transplantable lymphoblastoma in young chicks fed a mixed diet treated with ferric chloride in ether to destroy vitamin E. It may be noted, however, that the domestic fowl is prone to spontaneous manifestation of leucemic disease. Furthermore, our own experiences in experiments with rats indicate that ferric chloride treatment of mixed diets does not consistently destroy all traces of vitamin E present, especially when the fat content of the diet is low. The experiments of Davidson[2, 3] suggest that an excess of vitamins, especially liberal additions of vitamin E, decreases the incidence of tar carcinoma in mice. However, in repeating these studies Cameron and Meltzer[4, 5] observed only a delay in the appearance of the growth, which they attributed to the larger size and increased resistance of the vitamin-fed animals rather than to a specific vitamin effect. These

---

* This investigation was aided by a grant to Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

[1] Adamstone, F. B., *Am. J. Cancer*, 1936, **28**, 540.

[2] Davidson, J. R., *Canadian Med. Assn. J.*, 1934, **31**, 486.

[3] Davidson, J. R., *Canadian Med. Assn. J.*, 1935, **32**, 364.

[4] Cameron, A. T., and Meltzer, S., *Am. J. Cancer*, 1937, **30**, 55.

[5] Cameron, A. T., and Meltzer, S., *Am. J. Cancer*, 1937, **30**, 70.

conclusions are in accord with the observations that tumors produced in susceptible mice by painting the skin with 3-4 benzypyrene[6] or with methylcholanthrene[7] are in no way influenced by the vitamin E content of the diet. Furthermore, the development of the Ehrlich carcinoma in mice is not influenced by frequent subcutaneous injections of wheat germ oil[8] and neither deficiency nor excess of vitamin E in the diet of the rat bears any relationship to development of the Jensen sarcoma.[9, 10] It should be emphasized that the studies reported have been concerned only with the influence of the absence or presence of vitamin E upon a single generation of the neoplasm concerned. It was felt that prolonged maintenance of a neoplasm in vitamin E-deficient rats, by successive implantations, would afford more convincing evidence concerning any possible relation of this vitamin to tumor growth; and perhaps test the validity of the hypotheses that vitamin E may be essential to the maintenance of nuclear function and structure in rapidly dividing cells[11, 12] or related to the control of mitotic activity of the cell nucleus.[13]

The Walker 256 mammary carcinoma was maintained by successive implantations, at intervals of 30 to 45 days,† in the following groups of rats: (1) male and female rats fed a stock diet of commercial dog biscuit, (2) female rats fed an E-deficient diet supplemented with 10 drops of wheat germ oil, daily, beginning at the time of tumor inoculations, and (3) female rats fed an E-deficient diet and proven to be suffering from sterility due to lack of vitamin E. The vitamin E-deficient diet contained casein (22%), starch (54%), lard (20%), salts (4%), supplemented with 1 g of brewer's yeast and 6 drops of cod liver oil, daily. A total of 98 E-deficient and 54 control rats was used.

Tumor tissue obtained from the donor rat while under light anesthesia was cut into small fragments and transplanted into the subcutaneous tissue of the abdominal wall of the lightly anesthetized recipient. Aseptic technic was used throughout the operative procedure. During the first few series of transplants the trocharneedle method was used. Subsequent implants were made by inserting a small piece of the inoculum into the subcutaneous tissue through a small skin incision subsequently closed with a metal skin clip. The latter method gave a decidedly higher percentage of "takes" than the former, which may have been due in large measure to other improvements in the technic of inoculation. The size of the inoculum was kept as constant as possible. Schrek[14] has observed that variation in the size of the inoculum produces a significant effect upon the percentage of "takes" and the length of the "latent periods", but has little effect upon the growth-constant of the tumor.

---

[6] Haddow, A., and Russell, H., *Am. J. Cancer,* 1937, **29**, 363.

[7] Carruthers, C., *Am. J. Cancer,* 1939, **35**, 546.

[8] Engel, P., *Z. f. Krebsfor.,* 1933, **39**, 148.

[9] Marchesi, F., *Riv. di Patol. Sper.,* 1933, **11**, 396.

[10] Zagami, V., *Riv. di Patol. Sper.,* 1933, **11**, 381.

[11] Mason, K. E., *Am. J. Anat.,* 1933, **52**, 153.

[12] Juhász-Schäffer, A., *Ergeb. d. Inn. Med. u. Kinderh.,* 1933, **45**, 129.

[13] Adamstone, F. B., *Science,* 1934, **80**, 450.

† Depending upon the duration of growth and onset of central necrosis in the tumors of donor rats.

The animals were observed daily after the inoculation to determine the earliest signs of growth. The number of days elapsing between the time of the inoculation and the day on which a nodule was first palpable was considered the latent period, in accordance with the definition of Ottensooser.[15] The latent period was further determined by the method of Schrek,[16] whereby the interception of the linear growth curve on the abscissa determines the duration of the latent period. Both methods gave essentially the same result, except that the linear curve intercepted the abscissa about one day before a nodule was palpable. The latter observation remained constant throughout these studies. Measurements of the tumors were made every 3 or 4 days. The rate of growth of the tumor was evaluated by the method of Schrek,[16] modified to the extent of using the arithmetic mean of 2 or more diameters of the tumor instead of the geometric mean of 3 diameters. Both criteria were carefully compared and were found to give essentially the same results. From the data obtained it was possible to express the growth rate of the tumor in centimeters per day.

During 8 months maintenance in control and vitamin E-deficient rats through 6 successive transplantations the tumor exhibited no changes in its rate of growth. The percent of takes and the latent periods of the tumor were essentially the same in the 2 groups of animals (Table I). The tumor was subsequently maintained only in the E-deficient rats. However, at the 12th successive implantation the tumor was successfully inoculated into 14 deficient rats, 7 of which were given moderately large doses of vitamin E beginning at the time of inoculation. The growth rate and other characteristics of the tumor were practically identical in the two groups, and indistin-

---

[14] Schrek, F., *Am. J. Cancer*, 1936, **28**, 364.

[15] Ottensooser, R., *Z. f. Krebsfor.*, 1930, **30**, 553.

[16] Schrek, F., *Am. J. Cancer*, 1935, **24**, 807.

TABLE I.
Growth of the Walker 256 Mammary Carcinoma When Maintained for 8 Months in Control and Vitamin E-deficient Rats.

	Dietary treatment of rats		
	Stock diet (Controls)	E-deficient diet: supplemented with vitamin E (Controls)	E-deficient diet
Successive transplantations	6	6	6
No. of inoculations	44	54	61
No. of "takes"	19	19	28
% of "takes"	43	35	46
Latent period (avg) in days	4.5	4.25	4.3
Growth-constant (avg) in cm per day	0.130	0.135	0.133

guishable from those observed during the first 6 transplantations. Nor was any significant change observed after the tumor was carried through another 10 successive implantations in E-deficient rats, at the end of which time it had been maintained for 2½ years in E-deficient rats by means of 22 successive implantations.

Several pieces of the tumor from each rat were fixed in Zenker and prepared for routine histological study. Representative tumors from each series were fixed in corrosive-sublimate-acetic and stained according to the method of Ludford[17] for special study of the nuclear chromatin in normal and malignant cells. No significant differences were observed in either the histological organization or the cytological characteristics of the tumors removed from control and E-deficient rats.

In an additional series of studies an attempt was made to produce the transplantable intraäbdominal tumors observed by Rowntree and his associates[18] in rats fed an ether-extracted wheat germ oil subjected to treatment with sodium hydroxide. Daily doses of 1 cc of an oil prepared according to the method used by the latter investigators were fed to normal stock rats for periods of 128 to 214 days with no result. Bioassay of the oil revealed an exceedingly low concentration of vitamin E. Nor were any neoplasms observed in rats killed from 5 to 7 months after having received, over a period of 7 weeks, a daily intake of vitamin E equivalent to more than 2000 times the minimal daily requirement of the female rat.‡ These observations are in accord with those of numerous other investigators.[19–26]

---

[17] Ludford, R. J., *Proc. Roy. Soc. London, Ser. B*, 1928, **102**, 397.

[18] Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359.

‡ In the form of a molecular distillate of wheat germ oil, for which we are indebted to Dr. R. C. Sherwood, Research Laboratories, General Mills, Inc., Minneapolis.

The experimental data presented offer rather convincing evidence that vitamin E is in no way related to the induction of neoplastic growths, or to the growth and morphologic characteristics of the Walker 256 tumor in the rat. The fact that the latter tumor is not affected by prolonged existence in an environment devoid of vitamin E does not necessarily invalidate the hypothesis that vitamin E may be peculiarly necessary for maintenance of nuclear structure and function in rapidly proliferating tissues, but suggests that if any such relationship does exist it must be concerned with the more complex processes of cellular maturation and differentiation rather than with simple mitotic activity which represents the predominant type of cellular activity in the growth of neoplasms.

Summary. Maintenance of the Walker 256 mammary carcinoma in vitamin E-deficient rats for a period of 2½ years, by 22 successive implantations, produced no demonstrable alteration in the rate of growth, transplantability, histologic organization, or cytologic characteristics of the tumor.

Normal rats fed concentrates of wheat germ oil failed to show the neoplastic growths observed by Rowntree and his associates.

---

[19] Bacharach, A. L., *Nutr. Abst. and Rev.*, 1938, **7**, 811.

[20] Day, H. G., Becker, J. E., and McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 21.

[21] Carruthers, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 107.

[22] Halter, C. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 257.

[23] Demole, V., *Z. f. Vitaminforsch.*, 1939, **8**, 341.

[24] Evans, H. M., and Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 318.

[25] Dingemanse, E., and van Eck, W. F., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 622.

[26] Auchincloss, R., and Haagensen, C. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 703.

11204 P

Factors Determining Frequency Distribution of Lesions Induced by Papilloma Protein and Vaccinia Virus.*

W. Ray Bryan† *and* J. W. Beard.

From the Department of Surgery, Duke University School of Medicine, Durham, N. C.

Parker[1] has presented statistical evidence for the concept that the frequency distribution of lesions with vaccinia virus is related to the chance distribution of virus particles in the inocula. This was based on the resemblance of the observed frequency to the theoretical Poisson frequency to be expected if a single virus particle causes infection. Similar studies on the purified papilloma protein[2] have shown that results with this virus follow this theoretical expectation as well as did those with vaccinia virus. However, consistent deviations of observed from theoretical results at the extremes of the frequency distributions, and the finding of about 100,000,000 papilloma protein molecules in the dilution giving the 50% point (where only 0.69 particles should have been according to this concept) have led to critical examination of Parker's data and interpretations. Using the procedure recommended by Haldane[3] for determining χ^2, the fit of observed to theoretical frequencies in Parker's data was good in 2 experiments and improbable in 2.

On the basis of these inconsistencies other relations were sought to explain the character of results with vaccinia virus and the papilloma protein. It has seemed that a more logical interpretation and one more consistent with experimental results is that the observed frequency is an S shaped dose-response curve determined by variations in host resistance. If host variation follows a normal distribution, response to logarithmic doses will be S shaped and will be constant for a given material and population of test animals. Such a curve would represent an integrated frequency curve which can be placed in straight line form by plotting normal equivalent deviation against logarithm of dose.[4]

---

* This work was supported by a grant from the National Cancer Institute.

† Research Fellow, National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

[1] Parker, R. F., *J. Exp. Med.*, 1938, **67**, 725.

[2] Beard, J. W., Bryan, W. R., and Wyckoff, R. W. G., *J. Infect. Dis.*, 1939, **65**, 43.

[3] Haldane, J. B. S., *J. Hygiene*, 1939, **39**, 289.

[4] Burn, J. H., *Biological Standardization*, Oxford University Press, London, 1937; Gaddum, M. A., *Special Report Series Medical Research Council*, London, No. 183, 1933.

The data of Parker's experiments[1] and those obtained in the present work with the papilloma protein have been analyzed from this point of view. It was found that in all 4 of Parker's experiments the observed points fell about a straight line with a high degree of probability. This is illustrated in Fig. 1 for which the data of Parker's Exp. 4 were used. In Fig. 1 (a) is shown the relation of the observed points to the straight line drawn by the method of least squares. The Poisson curve has been drawn from the theoretical values given by Parker, and the deviation of observed points from the curve is shown in Fig. 1 (b).

The response to the papilloma protein likewise is linear until the 22nd day of the incubation period (which usually lasts about 30 days). Due probably to acquired resistance developing at about this period, as shown by actual retrogression of warts in some animals, there occurs a break in the line increasing progressively to the 32nd day. The response at this time assumes a character compatible with the concept that it is determined by change in the level of host resistance, from that of natural resistance to that of the total of natural

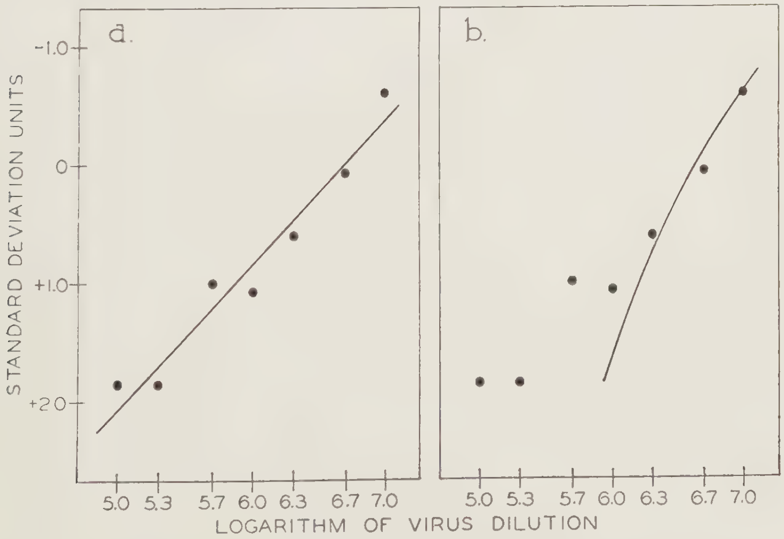


Fig. 1.

The points represent observed percentage response to vaccinia virus in Parker's Exp. 4 expressed as normal equivalent deviation, that is, the deviation on a normal frequency curve to which the percentage is equivalent. The deviation is given in standard deviation units.

(a) Comparison of observed points with the straight line drawn by the method of least squares.

(b) Comparison of observed points with the curve determined from theoretical values given by Parker on the assumption that a single virus particle infects.

plus acquired resistance. This possibility will be illustrated in detail in a forthcoming publication.

These results indicate that the character of the dose-response curves obtained with these viruses is determined, not by chance presence or absence of virus particles in inocula, but by variations in host resistance.

11205

Lack of Demonstrable Androgenic Activity of Desoxycorticosterone Acetate.*

R. R. GREENE AND M. W. BURRILL. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Crystalline desoxycorticosterone has been isolated from the adrenal cortex.[1] Manifold biological activities have been attributed to this substance. It has a potent cortinomimetic activity[2] and has been shown to be a fairly effective progestational substance.[2-5] Salmon[6] has stated that it has an estrogenic effect on the human vagina. Robson[4] has reported that desoxycorticosterone will directly antagonize and nullify the effects of simultaneously administered estrogen on the vagina of the castrate mouse. These last two observations are contradictory and could only be reconciled by the assumption that there is a marked difference between the human and the mouse in their reactions to this compound.

The present authors have offered direct evidence that the adrenals of immature castrate male rats, 26 to 31 days of age, are capable of producing an androgenic substance.[7, 8] In this paper it was noted that adrenosterone[9] and progesterone[10] had been isolated from the

---

* Supported in part by a grant from the Josiah Macy, Jr., Foundation.

[1] Reichstein, T., and Euer, J. V., *Helv. chim. acta*, 1938, **21**, 1197.

[2] Meischer, K., Fischer, H. W., and Tschopp, E., *Nature*, 1938, **142**, 435.

[3] Wells, J. A., and Greene, R. R., *Proc. Am. Phys. Soc.*, 1939, p. 236.

[4] Robson, J. M., *J. Physiol.*, 1939, **96**, 1.

[5] Van Heuverswyn, J., Collins, V. J., Williams, W. L., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 552.

[6] Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 515.

[7] Burrill, M. W., and Greene, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 327.

[8] Burrill, M. W., and Greene, R. R., *Endocrinology*, in press.

[9] Reichstein, T., *Helv. chim. acta*, 1936, **19**, 223.

[10] Beall, O., and Reichstein, T., *Nature*, 1938, **142**, 479.

adrenal, and that both were androgenic.[9, 11, 12] It was also suggested that because of the chemical similarity between progesterone and desoxycorticosterone the latter might also prove to be androgenic. (See also Hamilton and Golden.[13]) However, the following evidence shows that the foregoing speculation was erroneous.

Procedure. Fifteen male rats 31 days of age were castrated. Eight of these served as untreated castrate controls. The other 7 were given desoxycorticosterone acetate† subcutaneously in oil in daily doses varying from 1.0 to 2.5 mg. Treatment was administered for 8 days. The total doses varied from 8.0 to 20.0 mg. All animals were killed on the day following the last treatment, at 39 days of age. Ventral prostates were removed, fixed in Bouin's fluid and prepared for histological study. All the prostates, both from treated and control animals, showed castration atrophy with no evidence of androgenic stimulation.

Insufficient dosage per gram of animal was considered as a possible reason for the negative results obtained in the above experiment. It was therefore decided to use younger animals. The ventral prostate of the younger animal, however, may remain in the functional state for a limited period after castration (up to 31 days of age). It has been demonstrated, however, that if the animal is adrenalectomized as well as castrated, atrophy of the ventral prostate immediately ensues.[7, 8] Accordingly 15 males, 20 or 21 days old, were adrenalectomized and castrated. Six were treated with desoxycorticosterone acetate. All animals were killed on the 6th day after operation. At autopsy the ventral prostates were removed and prepared for histological study. At this time a careful search was made for adrenal tissue. Three of the untreated animals were "slips" as judged by their daily weight gains and the recovery of adrenal tissue at autopsy. One untreated animal died on the 4th post-operative day. These animals are not included in the results.

In accordance with our previous observations, the 5 untreated adrenalectomized castrates showed complete prostatic atrophy. Four animals were given 4.0 mg of desoxycorticosterone acetate daily subcutaneously in oil (total dose of 20.0 mg). The prostates of these animals also showed castration atrophy. Because of the low solubility of this substance in oil, it was impossible to give higher doses with this solvent to this sized rat. The substance is more soluble in

---

[11] Lamar, J. K., *Anat. Rec.*, 1937, **70**, Suppl. p. 45.

[12] Greene, R. R., Burrill, M. W., and Ivy, A. C., *Endocrinology*, 1939, **24**, 351.

[13] Hamilton, J. B., and Golden, W. R. C., *Endocrinology*, 1939, **25**, 737.

† The desoxycorticosterone acetate used in these experiments was kindly furnished by Ciba Pharmaceutical Products, Inc.

propylene glycol which was the vehicle used to administer daily doses of 10.0 mg (total dose 50.0 mg) to 2 other animals. The prostates of these animals were also completely atrophic.

In none of the 13 animals treated with 1.0 to 10.0 mg of desoxycorticosterone acetate daily was there any evidence of androgenic stimulation. The histological test used is based on the fact that androgens prevent regression of the ventral prostate after castration. This is the most sensitive of mammalian tests for androgenicity.[14] Therefore, we feel justified in concluding that desoxycorticosterone acetate is not androgenic in the rat.

11206 P

A Surgical Experiment in Changing the Refraction of the Eye.

MEYER WIENER. (Introduced by E. A. Graham.)

From the Laboratory, Oscar Johnson Institute, Department of Ophthalmology, Washington University School of Medicine, St. Louis, Mo.

The eye has been shortened surgically. There has been no attempt to lengthen it by surgical means. Mueller[1] reported a complicated operation for shortening the axis, by first performing a Kroenlein and then removing an eliptical segment of sclera from the exposed temporal side, 20 mm long and 8 mm to 10 mm wide, placing 5 scleral sutures and then puncturing the choroid before tying them to avoid injury. The object was the cure of detached retina, on the assumption of disproportion of the shrinking retina and the ocular shell. Recently, Lindner[2] proposed cutting the superior rectus muscle, excising an eliptical piece of sclera, the long axis equatorially, stitching the scleral wound so as to avoid injuring the choroid and sewing the severed muscle and conjunctiva back in place; later, doing the same thing below, and in turn, the two sides. In other words, 4 stages. Barkan[3] recently exhibited a moving picture of an operation where a strip of sclera was removed equatorially, after first severing the 4 recti muscles. This operation, most formidable, required over 4 hours to perform.

The method I am presenting is based on the principle that if a body with a given content, is shortened equatorially, it must expand

---

[14] Moore, C. R., Price, D., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 71.

[1] Mueller, L., *Beitrage zur Augenheilk.*, May-June, 1903, 459.

[2] Lindner, K., *Z. f. Augenheilk.*, 1933, **81**, 277.

[3] Barkan, H., moving picture shown at St. Louis University School of Medicine, October, 1939.

axially; and vice versa. It seems not to be necessary to excise, for example, a strip of sclera the entire length of the axis of the eye when one wishes to lengthen the eye by cutting out segments from the sclera longitudinally. In a dog's eye, which closely approximates the size and shape of the human eye, if one excises an eliptical segment of sclera 9 mm long and 1.5 mm wide from 4 opposite sides of the eye and brings the edges together by means of 3 sutures each, the eye will be lengthened approximately 2.5 mm to 3.0 mm. The refraction is changed from about normal to between 8 and 9 diopters minus. Mathematically, the distance from the cornea to the retina is increased by 1.27 times the width of one of the segments removed. Actually, it seems to be quite a bit more than this.

In shortening the eye, the decrease is 0.636 times the width of the segment removed, or about half as much as in the lengthening process. The segments are removed between the recti muscles, thus obviating the necessity of cutting the muscles.

The segments for shortening the eye can also be taken from the sides, between the muscles. The eye cannot be shortened, however, nearly as much as it can be lengthened by this method for obvious reasons. If indicated, there could be no objection to a later, additional, similar operation. Shortening the dog's eye by this method produces a scant 1 mm difference, or a refractive change of about 3 diopters.

Experiments are being conducted on live rabbits, which give the same changes in the refraction as were found in operating on excised eyes. Many eyes were spoiled before the technic could be perfected, since conditions on the living eye, such as changes in position, large size of the cornea, different arrangement and distribution of the long ciliary vessels and thickness of the sclera make it much more difficult to perform than on the human eye. Should one puncture the choroid, the results of the lengthening operation are apt to be spoiled, for the reason that the contents of the eye will no longer be the same, and consequently the axis is not forced to increase to the same extent. It makes very little, or no difference if this happens during the shortening process.

There are many problems involved which may alter or influence the ultimate success or failure of the procedure. One is that of increased intra-ocular tension. Will it gradually subside; and if it does, will that alter the physical aspect of the lengthened or shortened globe? Will the nourishment of the internal tissues be affected? Will the cut edges of the sclera eventually pull away? These are just a few of the aspects which must be considered.

The problem gives promise of developing into a valuable, practical surgical procedure.

11207

Quantitative Growth of Skin and Subcutaneous Tissue in Relation to Human Surface Area.

HARRY A. WILMER. (Introduced by Richard E. Scammon.)

From the Department of Anatomy and the Graduate School, University of Minnesota, Minneapolis.

The body is enveloped in skin in immediate contact with the external environment. But the developmental changes in the mass of this covering and of the tela subcutanea immediately under it have received little study.

Bischoff[1] presented a carefully prepared study of the weights of the skin, fat, and various parts of the body of a premature and 2 full-term infants, an adolescent child, and 2 adults. Various later authors (Brüel, Dursy, Volkmann and Wacherman, quoted from Welcker and Brandt,[2] von Liebig,[3] Vierordt,[4] Jackson,[5] and Roe[6])

TABLE I.
Age Changes in Amount of Skin and Tela Subcutanea from 5 Fetal Months to Maturity.

Age	5 lunar months*	Newborn*	Maturity		
Sex	M + F	M + F	M	F	M + F
No.	—	—	13	2	15
Mean age (yrs)	—	—	37	39	37+
Mean stature (cm)	22.9	50.5	168.8	159.5	—
Mean wt (kg)	0.261	3.000	57.300	50.147	—
Calculated surface area (cm^2)	347.8	2,102.0	16,273	14,953	15,613
Mean skin wt (g)	10.7	187.2	3,289.7	3,000.0	3,145.0
Mean tela subcutanea wt (g)	2.0	392.0	6,604.0	11,897.0	9,250.5
Calculated skin wt per cm^2 body surface (g)	0.031	0.086	0.202	0.200	0.201
Calculated tela subcutanea wt per cm^2 body surface (g)	0.006	0.200	0.406	0.796	0.601
Ratio of skin wt to tela subcutanea wt†	5.35	0.48	0.50	0.25	0.34

* Age calculated from length according to the formula of Scammon and Calkins.[9]

†The ratio of skin to tela subcutanea was computed from the values for these structures given in 10 of the thirteen cadavers measured.

---

[1] Bischoff, E., *Z. f. rat. Med.*, 1863, Ser. 3, **20**, 75.

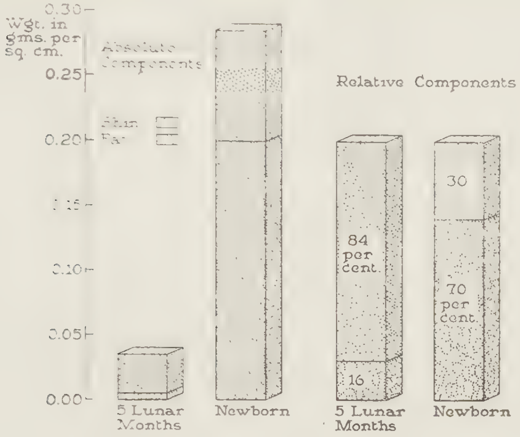
[2] Welcker, H., und Brandt, A., *Arch. f. Anthrop.*, 1903, **28**, 1.

[3] von Liebig, G., *Arch. f. Anat. u. Physiol.*, Jhrg. 1874, 96.

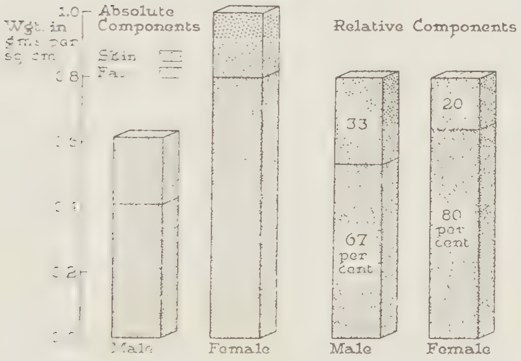
[4] Vierordt, H., *Anatomische, physiologische und physikalische Daten und Tabellen*, 3 Aufl., Jena, Gustav Fischer, 1906, p. 42 *et seq.*

[5] Jackson, C. M., *Am. J. Anat.*, 1909, **9**, 119.

[6] Roe, H. E., *Anat. Rec.*, 1933, **55**, 127.



Prenatal Growth



Adults

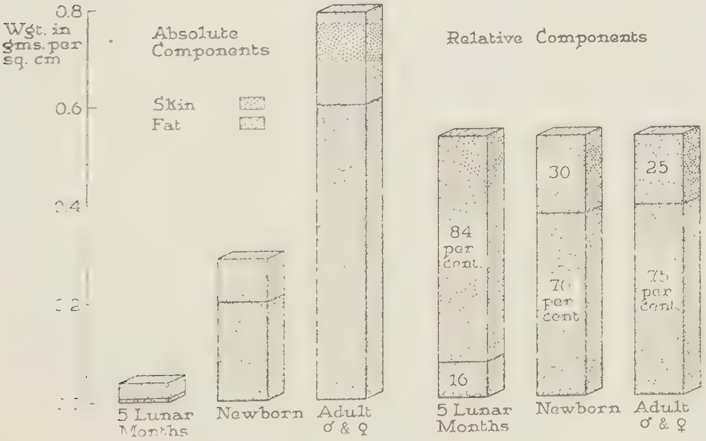


Fig. 1 (top); Fig. 2 (center); Fig. 3 (bottom).

have studied various parts of the body, including quantitative skin changes.

The total weight of the skin and the tela subcutanea at 5 lunar months, at birth, and in maturity (in males, females, and both sexes) has been collated from these data. The mean skin weight and tela subcutanea weight per square centimeter were calculated by the surface formulae of Scammon and Klein[7] and of Boyd,[8] and the body length-body weight formulae of Scammon and Calkins.[9] These data are shown in Table I. They are illustrated in histographic form in Figs. 1, 2, and 3.

Between 5 and 10 lunar months there is a very striking change. The skin increases about 17.5 times in absolute weight, while the tela subcutanea increases about 196 times. But the surface area of the body increases to such a degree during this time interval that the tela subcutanea increases but a little less than 35 times in terms of grams per square centimeter of surface.

In the adult, when both sexes are considered, it is found that the ratio of skin and tela subcutanea per square centimeter of surface is almost identical with that at birth. However, the ratio of the tela subcutanea in adult females alone is higher than in the newborn. There is comparatively little change in the proportion of skin to tela subcutanea in the 2 sexes.

The distribution of fat in the fetus and newborn infant (Berg[10]) is remarkably different from that in the adult. With this in mind, we might think of the changes in tela subcutanea as largely those of distribution, the relative quantity remaining approximately the same.

---

[7] Scammon, R. E., and Klein, A. D., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 461.

[8] Boyd, E., *The Growth of the Surface Area of the Human Body*, Minneapolis, University of Minnesota Press, 1935, p. 103 *et seq.*

[9] Scammon, R. E., and Calkins, L. A., Proc. Soc. Exp. Biol. and Med., 1923, **20**, 353; Proc. Soc. Exp. Biol. and Med., 1924, **21**, 549; Proc. Soc. Exp. Biol. and Med., 1924, **22**, 157.

[10] Berg, W., *Z. f. Morphol. u. Anthrop.*, 1911, **13**, 205.

11208 P

Tetanus Toxin Production on a Simplified Medium.

J. HOWARD MUELLER AND PAULINE A. MILLER.

*From the Department of Bacteriology and Immunology, Harvard University Medical School, Boston, Mass.**

The routine preparation of tetanus toxin for the purpose of antitoxin production, or for conversion into toxoid, appears to offer difficulties similar to those formerly encountered in the case of diphtheria toxin, although perhaps in lesser degree. Active immunization against tetanus by means of toxoid, now coming into general use, demands that an attempt be made to place the manufacture of this important biological product on as satisfactory a basis as it has now been possible to reach in the case of diphtheria toxin.[1, 2] The traditional explanation for failures and irregularities with the latter, namely the types of peptones and meat infusions used in the medium, has been shown to be completely erroneous, although still held to be applicable in dealing with tetanus. In this country, Witte, or the very similar Berna peptone is generally held to be indispensable, together with veal infusion. Elsewhere, enzymatic digests are employed, often with the addition of even more complex materials.

The writers have undertaken a study of the nutritional requirements of the tetanus bacillus and a parallel study of factors influencing the production of its toxin. Since an exact definition of the substances essential to growth may well prove to be a time-consuming matter, it seems worth while to report at this time the production of potent toxin on a simplified, but by no means chemically defined medium, and to indicate that the concentration of iron in the medium, as in the case of diphtheria, is at least one of the controlling factors.

As a basis for experiment we have employed the casein acid hydrolysate medium described by one of us[2] for the production of diphtheria toxin. This medium itself fails to support growth of any of the tetanus strains investigated, as was to be expected, but when it is enriched by the addition of tryptophane, glucose and 1% of a commercial liver extract, growth occurs with all the strains which have been tried. The liver extract employed is the crude 95% alco-

---

* Aided by a grant of the Commonwealth Foundation.

[1] Pappenheimer, A. M., Jr., and Johnson, S. J., *Brit. J. Exp. Path.*, 1936, **17**, 335.

[2] Mueller, J. H., *J. Immunol.*, 1939, **37**, 103.

hol precipitate fraction used in the treatment of pernicious anemia, and the writers are indebted to the Lederle Laboratories, Inc., and to Dr. Y. Subbarow of the Department of Biochemistry, Harvard University Medical School, for a supply of the material.

Using a culture obtained from the New York State Department of Health at Albany, through the courtesy of Dr. Harold W. Lyall, we have regularly obtained toxin of considerable potency on the medium. Only small experimental lots have so far been produced. These have been tested after the addition of 0.5% phenol and prolonged centrifuging, but have not been filtered because of the likelihood of considerable loss through adsorption. We realize that this procedure is not beyond criticism.

Toxin killing mice in 4 days in a quantity of 0.000,01 cc has been obtained (about 100,000 M.L.D.). Tested on guinea pigs in quantities of 0.000,2, 0.000,1, 0.000,05 and 0.000,025 cc the same material caused death in 39, 50, 72, and 76 hours respectively, indicating approximately 50,000 M.L.D. for this species. Addition of $FeSO_4$ to the medium before inoculation (iron having first been removed as completely as possible by adsorption[1] on a calcium phosphate precipitate), resulted in progressively weaker toxin as the concentration was increased up to 0.3 mg Fe per liter. The effect of the iron was more pronounced when cultures were grown in well-filled Erlenmeyer flasks exposed to the air, than when strictly anaerobic conditions were employed.

The work is being continued in an effort to identify the components of the liver extract essential to growth of the tetanus bacillus, and to define the optimal conditions for toxin formation.

11209

Pyridine Derivatives and Other Compounds as Growth-Promoting Substances for Dysentery Bacilli.*

STEWART A. KOSER, ALBERT DORFMAN AND FELIX SAUNDERS.

From the Departments of Bacteriology and Parasitology and of Biochemistry, University of Chicago.

The activity of a number of pyridine derivatives as growth-promoting substances for dysentery bacilli has been reported by us.[1] More recently several additional compounds of special interest were secured and tested. These were: synthetic vitamin B_6, nicotinamide methiodide, pyrazine monocarboxylic acid, pyrazine 2,3-dicarboxylic acid, thiazole 5-carboxylic acid, thiazole 5-carboxylamide† and quinolinic acid.

Vitamin B_6 is a pyridine compound with the structure 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine.[2] It seemed of interest to determine whether this compound is capable of acting as a growth-promoting substance for dysentery bacilli when substituted for nicotinic acid or nicotinamide. Another pyridine compound of possible physiologic interest is nicotinamide methiodide which Karrer and Warburg[3] found could be reversibly oxidized and reduced, as can the coenzymes. The nicotinamide methiodide was prepared according to Karrer and coworkers[4] and identified by melting point. The preparation was recrystallized seven times from an alcohol-ether mixture.

Pyrazine monocarboxylic acid and pyrazine 2,3-dicarboxylic acid were included because of the recent report by Bills, McDonald and

---

* This work was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago and a grant from the Committee on Scientific Research of the American Medical Association.

[1] Dorfman, A., Koser, S. A., Reames, H. R., Swingle, K. F., and Saunders, F., *J. Inf. Dis.*, 1939, **65**, 163; Koser, S. A., Dorfman, A., and Saunders, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 311.

† We are indebted to the Research Laboratory, Merck and Company, for the supply of synthetic vitamin B_6, to Dr. C. E. Bills of Mead Johnson and Company for the pyrazine compounds and to Dr. F. C. Schmelkes of Wallace and Tiernan Products, Inc., for the thiazole preparations.

[2] Stiller, E. T., Keresztesy, J. C., and Stevens, J. R., *J. Am. Chem. Soc.*, 1939, **61**, 1237; Harris, S. A., Stiller, E. T., and Folkers, K., *ibid.*, **61**, 1242; Kuhn, R., and associates, *Ber.*, 1939, **72**, 305, 309, 310.

[3] Karrer, P., and Warburg, O., *Biochem. Z.*, 1936, **285**, 297.

[4] Karrer, P., Schwarzenbach, G., Benz, F., and Solmssen, U., *Helv. Chim. Acta*, 1936, **19**, 811.

Spies[5] that administration of these compounds produced a prompt improvement in the condition of pellagrins. The thiazole compounds are interesting because they are isoteric with nicotinic acid and nicotinamide. Schmelkes[6] has suggested that these isosteres may be interchangeable biologically.

All compounds were sterilized by filtration through sintered glass filters and added aseptically in a series of graded dilutions to a basal synthetic medium. This medium was the same as that employed in previous work[1] and consisted of 15 amino acids, glucose and inor-

TABLE I.

Compound	Molar concentrations	Growth-promoting effect for dysentery bacilli
Vitamin B_6	1×10^{-4} to 10^{-6}	No growth*
Nicotinamide methiodide	1×10^{-4} to 10^{-6}	” ”
Pyrazine monocarboxylic acid	1×10^{-3} to 10^{-6}	” ”
Pyrazine 2,3-dicarboxylic acid	1×10^{-3} to 10^{-6}	” ”
Thiazole 5-carboxylic acid	1×10^{-3}	Slow growth, 4 to 18 days, with 9 of 10 cultures; one culture no growth.
	1×10^{-4}	Slow, light growth, 10 to 21 days, with 3 of 10 cultures; other 7 no growth.
	1×10^{-5}	No growth.
Thiazole 5-carboxylamide	1×10^{-3}	Slow growth, 5 to 21 days, with 5 of 10 cultures; other 5 cultures no growth.
	1×10^{-4}	Slow, light growth, 7 to 21 days, with 3 cultures; other 7 no growth.
	1×10^{-5}	No growth.
Quinolinic acid, recrystallized (filtered)	1×10^{-4}	Good growth, 2 days.
	1×10^{-5}	No growth.
Quinolinic acid, recrystallized (heated)	1×10^{-4}	Good growth, 24 hours.
	1×10^{-5}	Light or good growth, 24 hours.
	1×10^{-6}	Negative or light growth, 24 hr; good growth in 48 hr.
Controls:		
Nicotinic acid	1×10^{-6}	Good growth, 24 hr.
	1×10^{-7}	Light growth, 2 to 3 days; heavier growth 4 days.
	1×10^{-8}	No growth or occasional delayed growth, 10 to 14 days.
Nicotinamide	1×10^{-7}	Good growth, 24 hr.
	1×10^{-8}	Very light growth.

* All culture tubes in which growth did not appear were held at 37°C for 14 days and in many instances for 21 days before being recorded as negative.

---

[5] Bills, C. E., McDonald, F. G., and Spies, T. D., *Southern Med. J.*, 1939, **32**, 793.

[6] Schmelkes, F. C., *Science*, 1939, **90**, 113.

ganic salts. Ten strains of dysentery bacilli belonging to the Flexner and Sonne subgroups were used. It was known from previous work (1) that these cultures either did not develop, or in a few instances, developed only to the extent of a light turbidity in the basal synthetic medium and (2) addition of small amounts of either nicotinamide or nicotinic acid to the synthetic medium exerted a pronounced growth-promoting effect so that cultures showed luxuriant growth within 24 hours.

The results are shown in Table I. Vitamin B_6, nicotinamide methiodide, and the two pyrazine compounds exerted no growth-promoting effect, even when used in relatively large amounts.

The two thiazole compounds showed a low order of activity; slow culture development appeared in the presence of relatively large amounts of these compounds while smaller amounts were negative. This finding confirms the report of Schmelkes[6] and is an interesting example of the replacement of a compound by an isostere. It will be noted, however, that on the basis of comparable effect the activity of thiazole 5-carboxylic acid is about one ten-thousandth or less of that of nicotinic acid. In this respect our results differ slightly from those of Schmelkes[6] who reported that thiazole 5-carboxylic acid was about one-tenth of one percent as active as nicotinic acid. In our experience thiazole 5-carboxylamide is slightly less active than its corresponding acid. Thus the difference in the order of activity between the thiazole amide and nicotinamide is even greater than that between the respective carboxylic acids.

Quinolinic acid was included because of the differing claims regarding its efficacy. It supported development of dysentery bacilli when used in relatively large amounts, 1×10^{-4} molar.[1] According to Lwoff and Querido[7] a similar concentration caused growth of *Proteus*. When *Staphylococcus aureus* was used no growth-promoting activity of quinolinic acid in concentrations up to 10^{-4} molar was observed either by Knight and McIlwain[8] or by Landy.[9] In canine blacktongue Woolley, *et al.*,[10] reported a single dose of 150 milligrams failed to alleviate the condition, but amounts of one gram given to pellagrins by Vilter and Spies[11] produced marked improvement.

A sample of commercial quinolinic acid was purified by repeated recrystallization. Tests of its growth-promoting activity for dysentery bacilli showed the activity decreased somewhat after the first recrystallizations but thereafter remained at a constant level. This low order of activity may perhaps be explained by decarboxylation at position 2 with formation of nicotinic acid. Heating a solution of the purified quinolinic acid in the autoclave for 30 minutes increased its activity approximately a hundred times.

---

[7] Lwoff, A., and Querido, A., *C. R. Soc. Biol.*, 1939, **130**, 1569.

[8] Knight, B. C. J. G., and McIlwain, H., *Biochem. J.*, 1938, **32**, 1241.

[9] Landy, M., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 504.

[10] Woolley, D. W., Strong, F. M., Madden, R. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1938, **124**, 715.

[11] Vilter, R. W., and Spies, T. D., *Lancet*, 1939, **2**, 423.

These results lead us to believe that the curative effect obtained by Vilter and Spies when they fed quinolinic acid to pellagrins might be due to the presence of small amounts of nicotinic acid. As far as we know, no one has determined the minimum amount of nicotinic acid necessary to relieve the symptoms of pellagra; but in one report it was stated that 60 mg per day would effect a cure.[12] Spies, Bean and Ashe report that as little as 40 mg per day may be effective.[13] On this basis it would be possible to effect a cure with "quinolinic acid" if it contained as little as 4-6% nicotinic acid. From our experience commercial quinolinic acid may well contain this much nicotinic acid. At least a part of the activity of quinolinic acid is due to an impurity since the potency of quinolinic acid decreases on recrystallization.

Summary. Vitamin B_6, nicotinamide methiodide, pyrazine monocarboxylic acid and pyrazine 2,3-dicarboxylic acid showed no growth-promoting effect for dysentery bacilli when added to a basal synthetic medium. Thiazole 5-carboxylic acid and its amide exerted a low order of growth-promoting activity. The use of purified quinolinic acid in 1×10^{-4} molar concentration was uniformly followed by culture development. This may have been due to gradual conversion to nicotinic acid. Heating the quinolinic acid solution greatly increased its activity. The efficacy of quinolinic acid in curing pellagra may be due to contamination with small amounts of nicotinic acid.

---

[12] Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. Am. Med. Assn.*, 1937, **109**, 2054.

[13] Spies, T. D., Bean, W. B., and Ashe, W. F., *Ann. Internal Med.*, 1939, **12**, 1830.

11210 P

Potassium Exchanges in Man in Brief Exercise.

ANCEL KEYS.

From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minnesota.

Severe muscular exercise produces a rise in plasma potassium (Ewig and Wiener,[1] Baetjer,[2] Keys[3]). A loss of potassium from the muscle associated with muscular activities can be proved under some conditions (Fenn[4]). Does it follow that muscular contraction always involves a loss of potassium from the muscle to the blood stream? Extreme or unphysiological conditions are required to give a positive answer from tissue analyses. Blood sampling studies have not ruled out the red cells as a source of potassium in the immediate changes resulting from brief exercise in man. Calculations from whole blood and plasma are inadequate because of analytical limitations.

Major changes of plasma [K] occur within the exercised region in man at a time when products of metabolism like lactate are only beginning to enter the plasma. Table I gives results from a typical experiment.

We have attempted to evaluate the rôle of the red cells in the K exchanges in young men exercised by violent running in place for 40 to 60 seconds. Blood samples were drawn under oil from an antecubital vein before and within a few seconds of the end of exercise. The heparinized samples were immediately centrifuged in long centrifuge tubes under oil at 2 to 5°C. After

TABLE I.

Plasma concentration in a young man after 55 seconds of running at top speed. Protein as g protein nitrogen per kg of water, K, Na, and lactate, as milliequivalents per kg H_2O. Times at approximate mid-point of withdrawal of sample.

Time after end of exercise, sec	Arterial plasma				Femoral vein plasma		
	Lactate	Protein	K	Na	Protein	K	Na
Rest	9	1.04	4.75	—	—	—	—
35	29	1.18	5.31	150.1	1.16	5.97	156.7
52	—	—	—	—	1.22	6.11	153.5
78	98	1.20	5.88	152.6	1.09	6.04	149.2
390	126	—	5.24	—	—	—	—

---

[1] Ewig, W., and Wiener, R., *Z. ges. exp. Med.*, 1928, **61**, 562.

[2] Baetjer, A. N., *Am. J. Physiol.*, 1935, **112**, 139.

[3] Keys, A., *Trans. Faraday Soc.*, 1937, **33**, 930.

[4] Fenn, W. O., *Physiol. Rev.*, 1936, **16**, 450.

TABLE II.

Analytical results from a typical experiment in which a young man exercised by standing running at top speed for 55 seconds. The "work" sample was drawn over the period between 20 and 70 seconds from the end of exercise. Concentrations of K, Total Base, and Na in terms of milliequivalents per kg of water of the cells and plasma respectively.

Condition	Cell vol., %	Water, g/kg		K		Total Base		Na
		Plasma	Cells	Plasma	Cells	Plasma	Cells	Plasma
Rest	43.2	923.9	666.4	4.95	135.7	154.4	164.6	148.8
Work	46.5	916.8	672.2	6.02	130.0	163.2	157.4	156.8
Δ	+3.3	—7.1	+5.8	+ 1.07	—5.7	+8.8	—7.2	+8.0
Δ, as % of Rest,	+7.6	—0.77	+0.86	+21.6	—4.2	+5.7	—4.4	+5.4

50 minutes at 2800 r.p.m. the oil was removed and the middle portion of the plasma taken for analysis. The remaining plasma and the top 0.5 cm of cells were removed and then the cells were sampled by syringe-pipettes (Krogh and Keys[5]). Plasma and cells were analyzed separately for water (drying at 105°C), total base (Keys[6]) and potassium (Hartzler[7]). Duplicates were run on all except K which was analyzed in triplicate. Results from a typical experiment are summarized in Table II.

Table II shows that [K] in the plasma rose 21.6%, but that apparently more than this change could be accounted for by loss from the red cells. More exact comparison requires allowance for the volumes of water and their alterations in the red cells and plasma. When correction is made for the water imbibed by the red cells, the net changes in K and total base per kg of red cell water become – 2.2 and – 2.9 m.eq. respectively, corresponding to losses from the red cells of 0.63 and 0.83 m.eq. per kg of whole blood. There is a gain of 1.07 m.eq. K per kg water by the plasma, or a gain of 0.52 m.eq. per kg of whole blood. The red cell loss of K is still greater than the plasma gain.

Further indication that no large net gain of whole blood K occurs immediately in brief exercise is found when the plasma concentration is considered in more detail. If the red cells were impermeable to K and there were a high K gradient from the muscle to the blood side of the capillary wall, then the water which is drawn to the muscles and red cells by osmotic forces in brief exercise should carry no K and the remaining plasma should have an elevated [K] from this reason alone. When correction is made for the water loss from the plasma on this basis the net increment of K is only + 0.27 m.eq. per kg of original whole blood.

---

[5] Krogh, A., and Keys, A., *J. Chem. Soc.* (Lond.) 1931, **1931**, 2436.

[6] Keys, A., *J. Biol. Chem.*, 1936, **114**, 449.

[7] Hartzler, E., *J. Biol. Chem.*, 1937, **122**, 19.

The findings discussed here are typical of experiments on 5 subjects. It is possible that the red cell-plasma K exchanges may have occurred *in vitro* in spite of our care. Such an effect could not, however, have masked any appreciable true gain of "foreign" potassium.

There is another possibility—new red cells low in potassium could have been added to the blood stream from the spleen or some other reservoir. It can be calculated that these "new" red cells would have to contain the extraordinarily low concentration of 105 m.eq. K/kg H_2O to account for the red cell values found.

There is no doubt that potassium enters the blood from the muscles when longer periods of exercise are used. It may be that even in these short periods of exercise K enters the blood from the muscle, but if so it must be removed elsewhere, perhaps in the liver, at an equal rate.

11211

Prevention of Histamine and Surgical Shock by Cortical Hormone (Desoxycorticosterone Acetate and Cortin) and Saline.

David Perla, David G. Freiman, Marta Sandberg and
Sidney S. Greenberg.

From the Laboratory Division, Montefiore Hospital, New York City.

It is well established from pathological and experimental studies that the suprarenal cortex plays a significant rôle in the mechanism of natural resistance to intoxications, poisons, shock and bacterial and protozoan infections.* The similarity between adrenal insufficiency and the condition of secondary shock has been emphasized by many investigators.[3, 4, 5] The therapeutic effect of cortical hormone in the prevention of anaphylactic shock in guinea pigs[6] and in shock due to intestinal obstruction in dogs[7] has been reported.

It was observed that parenteral administration of large amounts of physiological saline given some hours prior to the production of severe shock (by histamine) enhanced resistance in rats, enabling them to withstand about twice the lethal dose of histamine for normal animals (Perla[8]). In the present study the effect, both prophylactically and therapeutically, of the suprarenal cortical hormone with and without saline on resistance to shock was determined.

---

* This subject is reviewed by Perla and Marmorston.[1,2]

[1] Perla, D. and Marmorston, J., *Arch. Path.*, 1933, **16**, 379.

[2] Perla, D., and Marmorston, J., *Natural Resistance and Clinical Medicine*, Little, Brown and Co., Boston, Mass., 1940, in press.

[3] Sweet, J., *Am. J. M. Sc.*, 1918, **155**, 627.

[4] Swingle, W. W., Pfiffner, J. J., Vars, H. M., Bott, P. A., and Parkins, W. M., *Science*, 1933, **77**, 58 (includes review of literature).

[5] Zwemer, R. L., and Scudder, J., *Surgery*, 1938, **4**, 510.

[6] Wolfram, J., and Zwemer, R. L., *J. Exp. Med.*, 1935, **61**, 9.

[7] Heuer, G. J., and Andrus, W. de W., *Ann. Surg.*, 1934, **100**, 734.

Treatment of histamine shock in rats and mice with saline and cortin. A number of normal adult rats were each given an amount of histamine (1200 mg per kg of body weight) above the minimal lethal dose of this poison for our strain of rats (1000-1100 mg). Immediately thereafter they were divided into 4 groups. One group was given repeated injections of 5 cc of physiological saline intraperitoneally at short intervals, as indicated in Table I. A second group received adrenal cortical extract (Wilson) in 5 repeated doses at similar intervals of 15 to 30 minutes. A third group received both saline and adrenal cortical extract combined in a total amount of 5 cc at the same intervals. A fourth group was untreated. The results indicate that combined treatment was more beneficial than the use of saline or cortical extract alone (Table I). The experiment was repeated using a slightly higher amount of histamine (1300 mg per kg of body weight) with similar results.

A similar experiment was performed using young adult mice. In preliminary studies the minimal lethal dose of histamine for our strain was found to be about 400 mg per kg. Therefore, a series of mice were each given subcutaneously 600 mg of histamine per kg.

TABLE I.
Therapeutic Effect of Parenteral Saline and of Cortical Extract in Rats* on Histamine Poisoning. (Combined results of several experiments.)

Amt histamine acid phosphate per kg body wt	Untreated Rats		Saline† treated		Cortin‡ treated		Saline and§ Cortin treated	
	Total No.	No. survived	Total No.	No. survived	Total No.	No. survived	Total No.	No. survived
1200	10	4 (40%)	11	6 (55%)	12	9 (75%)	7	7 (100%)
1300	3	0 (all died within 15 min)	4	2 (2 died in 2 to 4 hr)	4	1 (3 died within 1 hr)	4	3 (1 died in 4 hr)

* The rats were of an inbred strain of Bartonella-free stock, 3 to 4 months of age.

† Physiological saline in amounts of 5 cc was given at intervals of 1, 15, 30, 45 and 75 minutes.

‡ Cortical extract (Wilson) in amounts of 2 cc was given at once and in 15 minutes, then 1 cc was administered at intervals of 30, 45, and 75 minutes.

§ The cortical extract as above was brought up to a total volume of 5 cc with saline and injected at the same intervals as in the other groups.

---

[8] Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 234.

They were divided into 4 groups. One group was repeatedly treated with parenteral saline, a second with cortin, a third with both saline and cortin, and a fourth was untreated. Details are given in Table II. Of mice treated with saline 23% survived; of those given cortin 30%. Of mice given both cortin and saline 71% survived. Only one of 14 untreated mice survived this dose of histamine.

Prophylactic effect of saline and desoxycorticosterone (Table III).

TABLE II.
Therapeutic Effect of Parenteral Saline and of Cortical Extract in Mice* on Histamine Poisoning. (Combined results of two experiments.)
(All mice were given 600 mg of histamine acid phosphate per kg body weight subcutaneously.)

Untreated		Cortin treated†		Saline treated‡		Cortin and Saline§ treated	
Total No.	No. survived	Total No.	No. survived	Total No.	No. survived	Total No.	No. survived
14	1 (7%)	13	4 (30%)	13	3 (23%)	14	10 (71%)

* The mice were of a special uniform "Swiss strain" inbred for susceptibility to viral and bacterial infection at the Rockefeller Institute by Dr. Webster and raised on the Carworth Farms. They were 20 to 22 g in weight.

† The adrenal cortical extract (Wilson) was given in amounts of 0.2 cc at intervals of 1 and 15 minutes; then 0.1 cc at intervals of 30, 45 and 75 minutes intraperitoneally.

‡ The physiological saline was given in amounts of 0.5 cc at the same intervals as in †.

§ The cortical extract as above was brought up to a total volume of 0.5 cc with saline and given at the same intervals as in †.

TABLE III
Prophylactic Effect of Parenteral Saline and Desoxycorticosterone on Resistance to Histamine in Rats*. (Combined data of several experiments.)

Amt of histamine acid phosphate per kg body wt	Untreated		Treated with Desoxycortico-sterone†		Treated with Saline‡		Treated with Saline and Desoxy.§	
	Total No.	No. survived	Total No.	No. survived	Total No.	No. survived	Total No.	No. survived
1200	8	0	4	1	—	—	—	—
1300	2	0	—	—	—	—	—	—
1400	2	0	4	0	2	1	2	2
1600	—	—	2	0	2	1	2	1
1800	—	—	—	—	4	2	2	2
2000	—	—	—	—	5	1	5	3
2200	—	—	—	—	2	1	4	1
2400	—	—	—	—	—	—	4	1
2600	—	—	—	—	—	—	2	0

* The rats were 3 to 4 months old, of Bartonella-free stock.

† The desoxycorticosterone acetate in oil was given subcutaneously in amounts of 5 mg 24 hours before the test and 3 mg 4 hours before the test.

‡ The physiological saline was given 24 hours and 4 hours before the test intraperitoneally in amounts of 15 to 20 cc.

§ In amounts as in † and ‡.

‖ A second animal survived 6 hours. The animals that succumbed died within one to 2 hours.

In these experiments 4 groups of young adult rats were used. In one group each rat received desoxycorticosterone acetate subcutaneously[†] 24 hours and again 4 hours before the test. In a second group each rat received saline intraperitoneally at the same intervals. In a third group each received both desoxycorticosterone acetate subcutaneously and saline intraperitoneally. In a fourth group the rats were unprepared. Four hours after the last injection, the animals were given histamine in amounts varying from 1200 to 2600 mg per kg. Desoxycorticosterone acetate alone afforded only slight protection. As previously demonstrated[8] the prophylactic administration of saline alone greatly enhanced resistance. More pronounced protection, however, was observed in the group of rats prepared both with saline and desoxycorticosterone acetate (an occasional animal surviving 2400 mg per kg).

The results were even more striking in mice (Table IV). Though saline alone was slightly more effective than desoxycorticosterone alone, the use of both protected the mice against histamine to a greater degree than either and raised their resistance to a level more than 5 times that of untreated mice.

Prevention of surgical shock in man. On the basis of these experiments a plan for the prevention of surgical shock was carried out in the preparation of a series of chronically ill patients for severe

TABLE IV.

Prophylactic Effect of Parenteral Saline and of Desoxycorticosterone on Resistance to Histamine in Mice.[*] (Combined data of 2 experiments.)

Amt of histamine acid phosphate per kg body wt	Untreated		Treated with Desoxycorticosterone[†]		Treated with Saline[‡]		Treated with Saline and Desoxycorticosterone[§]	
	Total No.	No. survived	Total No.	No. survived	Total No.	No. survived	Total No.	No. survived
750	5	0	—	—	—	—	—	—
1000	5	0	6	5	6	2	6	5
1200	—	—	6	4	6	2	6	5
1500	—	—	6	1	5	4	6	5
2000	—	—	3	1	6	1	6	3
2400	—	—	—	—	3	0	6	4

[*] The mice were of the same strain as in the previous experiments reported in this communication.

[†] The desoxycorticosterone acetate in oil was given subcutaneously in amounts of 1.5 mg 24 hours and 4 hours before the test.

[‡] The saline was given 24 hours and 4 hours before the test in amounts of 1 and 1.5 cc respectively, intraperitoneally.

[§] In amounts as in † and ‡.

[†] In the therapeutic experiments desoxycorticosterone could not be used because it is absorbed too slowly and at present is not available in a solvent permitting rapid absorption.

surgical procedures. From the fifth to the second day before operation, patients received daily 8 to 10 g of salt in divided doses in water, 3 liters of fluid by mouth, and 5 mg of desoxycorticosterone acetate intramuscularly. Two days prior to, on the day of, and on the day after operation intravenously 1000 to 1500 cc of physiological saline daily and 10 mg of desoxycorticosterone intramuscularly were given.‡ During 2 weeks after operation patients were given 5 mg of desoxycorticosterone daily for 5 days, then 5 mg every second day, as well as salt and high fluid intake by mouth. (General supportive measures such as a high protein, high vitamin diet with supplements of vitamin C were given where possible during the entire period.)

Table V shows the results in 12 consecutive instances in patients with chronic diseases in whom such operative procedures were performed as resection of colon or stomach, thoracoplasty and the like. None of these patients had any evidence of Addison's syndrome as determined by serum sodium, potassium and hematocrit determinations. In all instances the patients were strikingly benefited; there was no objective evidence of shock, blood pressure was maintained or elevated from 10 to 30 mm, the temperature in general returned to normal within 24 to 48 hours, post-operative exhaustion and toxemia were definitely lessened, complications did not occur and operative recovery appeared, to the surgeons concerned, to be more rapid than in their preceding surgical experience with similar conditions in our hospital.§ On the chest service the procedure has been adopted as a routine.

It is fully appreciated that this series of cases is in itself inadequate and that with further work our impressions may be modified.‖ However, the clinical results have been so striking that in view of the preceding experimental evidence we believe it justifiable to make a report as this time¶

Summary. In the prevention and treatment of histamine shock in rats and mice, parenteral saline combined with cortical hormone is

---

‡ Where only a short period of preoperative preparation is possible the desoxycorticosterone is increased up to 20 or 30 mg within 24 hours or cortical extract may be given intravenously in amounts up to 30 cc within this period in divided doses.

§ The only contraindications to the use of this treatment would appear to be cardiac decompensation, nephritis, and severe hypertensive states.

‖ The method is now being tried in two other large hospitals in the metropolitan area in addition to its continued use at Montefiore.

¶ We are indebted to Dr. Schwenk and Dr. Gilbert of Schering, to Dr. Oppenheimer of Ciba for supplying us with desoxycorticosterone acetate and to Dr. Klein of the Wilson Laboratory for the adrenal cortical extract.

TABLE V.

Use of Desoxycorticosterone Acetate and Saline in Prevention of Surgical Shock in Humans.

Case	Diagnosis	Pre-operative Duration in yrs	Blood pressure	Condition	Operative Type of operation	Anesthesia	Duration of operation	Pulse	Respiration	Blood pres.	Condition	Post-operative Temperature	Pulse	Respiration	Blood pres.	Condition
V.M. 39 yr	1. Tbc. lung 2. Tbc. Osteomyel.	7 1	120/88	Poor; anemic; bronchial stenosis, bronchiectasis; poor surg. risk	Spinal fusion (L_2-S_1) with tibial bone graft.	Gas—O_2 Ether	1½-2 hr	90-96	20-24	130/68	Excellent; no evidence of shock.	100.2-100.8	98-112	No change	108-140 / 86-90	Excellent: No asthenia Appetite good; Gained weight.
E.S. 49 yr	Ca. of rectum	2	108/60	Fair; toxic, 1st stage Lahey 1 mo previously; did poorly	Resection of rectum	Spinal	1-1½ hr	100-112	20	116/70	Excellent	100-101 (2 day 103)	110-96	No change	130-112 / 78-64	Good; asthenia less; appetite increased.
L.H. 25 yr	Pulm. tbc.	3	140/90	Good; rapid prep. within 2 hrs [Cortin (20cc) and 500 cc saline]	1st stage thoracoplasty	Gas—O_2	1 hr	80-11[illegible]	18-24	130/68	Alert, with sense of well-being	99-100.8	106-80	24-18	152-130 / 100-70	Excellent; 2nd stage done in 3 weeks
,, ,,	,, ,,	,,	,,	Good	2nd stage thoracoplasty	Gas—O_2	1 hr	112	24	168/88	Excellent	99.0-99.2	120-80	24-18	142-170 / 86-90	Rapid recovery; discharge in 2 weeks
S.W. 23 yr	Pulm. tbc. (bilat.)	3	102/68	Fair; status post pnthx. left Oleothx; left tbc. empyema. 1st stage—2 mo prev.; did poorly; needed transfusion	2nd stage thoracoplasty	Gas—O_2	1 hr	100	24	104/68	Same. Sense of well-being.	98-99	88-96	18-22	130-120 / 80-76	Excellent course in contrast to unprepared 1st stage

A.K. 20 yr	Extensive unilat. bronchiectasis	19	120/76	Fair; toxic	Attempted pneumonectomy.	Avertin Gas O$_2$	1½-2 hr	106	18-36	142/90	Op. not completed; mediastinal adhes.	100-102	112-128	24-36	112-146/80 - 96	Patient did well; temperature rise due to sputum retention
F.S. 43 yr	Ca. of colon (Splenic flexus)	3	122/64	Poor; anemia; marked asthenia and wt loss; febrile; diarrhœa 8 mos.	Resection with side to side anastomosis	Avertin plus inhalation anes.	2-3 hr	96-100	120/68	22	No signs of shock, Felt well. (transfusion hgb 48).	99.2-102	94-100	18-24	120/70	Retention of purulent matter in wound. Temp. normal after release. Out of bed in 2 weeks.
H.C. 39 yr	Pulm. tbc.	8	128/86	Fair; 1st stage done 5 weeks prev. with slow recovery and marked asthenia. (bl. pr.—96/30).	2nd stage thoracoplasty.	Gas—O$_2$	1 hr	104	118/86	20	Good	98.4-100.2	110-88	18-22	126-132/88 - 90	Little asthenia compared with 1st stage. No shock; rapid healing and recovery.
S.G. 55 yr	Cholelithiasis	1	140/80	Fair; prep. with vit. K. Also coronary dis. and mild hypertension.	Cholecystectomy	Spinal procain	1-1½ hr	84	134/68	26	Good	102-99.6	96-88	28-22	140-110/100- 68	For 5 days condition excellent. Evisceration with peritonitis (sutures removed too early). Rapid recovery.
H.B. 35 yr	Pulm. tbc. (bilateral)	11	104/68	Fair; (Gynecomastia and small testes). Low vital capacity. Marked fibrothorax.	1st stage thoracoplasty	Gas O$_2$	1 hr	96	118/88	28	No shock	98.6-99.2	96-88	28-20	140-112/100- 84	Patient felt well; appetite good; rapid recovery and healing.
J.T. 49 yr	Carcinoma of stomach	5	148/86	Fair; fibroid tbc. and arteriosel. heart disease	Partial gastric resection	Spinal	2½ hr	100	142/82	22	Temp. 100.6 After 3rd day normal	101-98	104-72	24-20	150/74	Excellent.
L.S. 26 yr	Pulm. tbc. (bilateral)	7	130/80	Fair; 1st stage 3 mos. prev. Became toxic, febrile and did poorly.	2nd stage thoracoplasty	Gas—O$_2$	1-1½ hr	88	134/92	22	Excellent	101-99.8	104-84	32-20	134-126/92 - 70	Excellent.

more effective than either alone. The prophylactic effect of this combined treatment in raising resistance of rats and mice to histamine shock is much more pronounced than the therapeutic effect after histamine is administered. Application of these experimental observations to the prevention of surgical shock in human beings is reported.

11212

Are Gonadotropic Hormones Destroyed While They Exert Their Action on the Ovary?

HANS SELYE.

From the Department of Anatomy, McGill University, Montreal, Canada.

The question whether hormones are inactivated by the organs on which they act is of fundamental importance for the interpretation of hormone action in general. Some authors regard hormones as catalysts, which would imply that they are not used up during their activity, while others believe that they are destroyed while they exert their action. This latter view appears to receive some support from observations showing that if hormone preparations are administered parenterally only a fraction if any of the injected material may be recovered from the excretions. The fact that removal of one ovary causes compensatory hypertrophy of the remaining gonad has likewise been looked upon as "an expression of the effect of the total amount of anterior pituitary hormone being available for one ovary."[1] However, neither one of these arguments is conclusive and little effort has been made, up to the present, to solve this problem by experimental investigation.

It is doubtless true that hormones are destroyed in the organism but this does not prove that they are used up by the organs upon which they act. Furthermore, the compensatory hypertrophy of an organ such as the ovary may not only be explained by assuming that more gonadotropic hormone is available for the ovarian remnant but might also be looked upon as resulting from increased gonadotropic hormone secretion by the hypophysis of the partially spayed animal.

In order to elucidate this problem, the following experiment was performed. Twenty "hooded" female rats were hypophysectomized at the age of 4 weeks. Ten of them were unilaterally ovariectomized at the same time. Immediately following the operation, one animal of each group received subcutaneous injections of one R.U. of a follicle-stimulating preparation (F.S.H.) and one R.U. of a luteinizing preparation (L.H.).* The second animal of each group received 2, the third. 3 units of each of these preparations and so on, each animal receiving one more unit so that the last animal in each group received 10 units of F.S.H. and 10 units of L.H. This treatment was repeated once daily for 5 days. On the sixth day, the animals were sacrificed and their ovaries were weighed.

---

[1] Adams, A. Elizabeth, *Proceedings of the Second International Congress for Sex Research,* 1930, p. 190.

It is obvious that the ovarian weight in these animals could only be determined by the amount of the injected gonadotropic hormone available for each ovary since in the absence of pituitary tissue, no compensatory gonadotropic hormone production was possible. If the ovary destroys gonadotropic hormone during maturation, the weight of one ovary in the unilaterally spayed females should be higher than that of one of the 2 ovaries in the animals possessing both their gonads. Table I shows, however, that this is not the case.

Since there are marked individual variations in the sensitivity to gonadotropic hormones, the parallelism between the dose administered and the resulting ovarian weight is not very close but it is easy to see that half of the combined weight of the 2 ovaries in the noncastrates corresponds approximately to the weight of the remaining ovary in the unilaterally spayed group at the various dose levels. Between one and 3 units, the increase in weight was not significant enough to draw conclusions although histologically some stimulation

TABLE I.

Units of F.S.H. and L.H. injected	Half of the wt of the 2 ovaries in non-spayed, mg	Wt of one ovary in unilaterally spayed, mg
1	7.5	7
2	8.5	7
3	9	7.5
4	14	12
5	8.5	11.5
6	12.5	12
7	18	13
8	17.5	17
9	14.5	18
10	22.5	22

---

* We are greatly indebted to the Løvens Kemiske Fabrik for the follicle-stimulating mare serum preparation "Antex Leo" and to the Ayerst, McKenna and Harrison Ltd. for the luteinizing human pregnancy urine extract "APL."

was quite obvious. However, if we compare the animals receiving 3 units with those receiving 6 or the group injected with 4 and 5 units with those injected with 8 and 10 units respectively, it becomes clear that if a certain amount of gonadotropic hormone is present, the resulting ovarian weight is considerably greater than if half of that dose is administered. From this it appears that if each ovary would use half of the injected hormone, the weight of a single ovary should be considerably larger in the group having but one gonad. This, however, is certainly not the case, in fact if we calculate the average weight of one ovary for the 10 different dose levels, it proves to be 13.3 mg in the non-spayed as compared with 12.7 mg in the spayed group which shows that there is no significant difference between the 2 series.

From these experiments it appears that the ovarian weight is determined by the mere presence of a certain concentration of gonadotropic hormones in the body fluids and that no significant destruction of this hormone takes place while it exerts its gonad-stimulating effect. Further experiments will have to show whether other hormones act in a similar manner. Great caution will have to be used, however, in extending such studies to the gonadal hormones. Hertz and Meyer[2] noted that removal of parts of the male genital tract does not increase the response of the remaining parts to testosterone nor does partial hysterectomy increase the response of the remaining uterine tissue to a standard dose of estrin. In view of the fact that these sex hormones act on many organs other than the genital tract,[3] it is difficult to draw any definite conclusions from these experiments because extirpation of part of the genital tract removes only an insignificantly small portion of the tissues upon which these hormones act.

Summary. In hypophysectomized and unilaterally ovariectomized rats, a certain dose of the gonadotropic hormones causes the same increase in the weight of the single ovary as this same amount causes in hypophysectomized animals possessing both their gonads. It is concluded that no significant destruction of the gonadotropic hormones takes place while they exert their action on the ovary.

---

[2] Hertz, Roy, and Meyer, Roland K., *Am. J. Physiol.*, 1938, **124**, 259.

[3] Selye, Hans, *Canad. Med. Assn. J.*, February, 1940, **42**, 113.

11213

Distribution of Melanophore-Dispersing Hormone in Anterior Lobe of Cetaceans and Armadillo.*

FRANCES K. OLDHAM, JULES H. LAST AND E. M. K. GEILING.
From the Departments of Pharmacology and Anatomy, University of Chicago.

The pituitary gland of cetaceans and of the armadillo has no discrete pars intermedia and the anterior and neural lobes are completely separated by connective tissue.[1-4] In these species, the melanophore-dispersing hormone is present in the anterior lobe,[3, 5, 6] whereas in most animals this hormone is ascribed to the pars intermedia. Hence, cells with a function similar to those of the pars intermedia of most other species are present in the anterior lobe of cetaceans and the armadillo. The present study was undertaken to determine whether this hormone is distributed uniformly throughout the lobe or whether it is concentrated in a particular region. It was felt that such an investigation might be of value in determining the distribution of the cells secreting the hormone, especially since no differential stain has been developed for these cells.

Material and Methods. 1. *Cetaceans:* Glands from the bottlenose dolphin *(Tursiops truncatus)*, the finback *(Balaenoptera physalis)* and the white *(Delphinapterus leucas)* whales were fixed in acetone. The connective tissue was stripped from the anterior lobes and blocks of tissue were cut from the extreme lateral portions of the lobe, from the medial postero-dorsal and medial antero-ventral surfaces, and, in the case of one porpoise, from the antero-dorsal surface (Fig. 1). The separated portions were cut into fine pieces and ground up in acetone. After removal of the acetone, the powders obtained were extracted with 0.25% acetic acid so that 1 cc contained the activity of 1 mg of acetone-desiccated powder.

2. *Armadillo (Dasypus novemcinctus):* Because of the small size of the gland in the armadillo, for each set of experiments, the separated portions from 4 animals were pooled, and treated with

---

* This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

[1] Wislocki, G. B., *Arch. Surg.*, 1929, **18**, 1403.

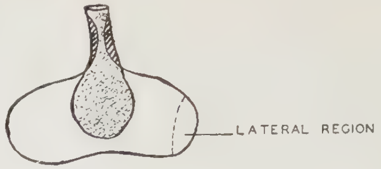
[2] Wislocki, G. B., and Geiling, E. M. K., *Anat. Rec.*, 1936, **66**, 17.

[3] Oldham, F. K., *Anat. Rec.*, 1938, **72**, 265.

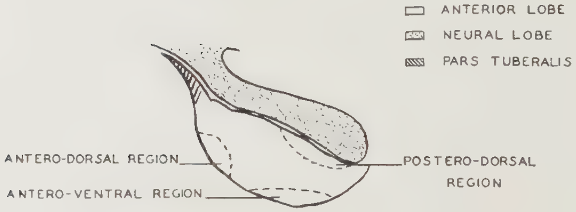
[4] Wislocki, G. B., *Anat. Rec.*, 1938, **70**, 451.

[5] Geiling, E. M. K., *Bull. Johns Hopkins Hospital*, 1935, **57**, 123.

[6] Valsö, J., *Klin. Wochenschr.*, 1934, **13**, 1819.



DORSAL ASPECT



MID-SAGITTAL ASPECT

FIG. 1.

acetone and 0.25% acetic acid, as described in the preceding paragraph. In the preliminary experiments, glands were separated into a central and a lateral portion; and an anterior and posterior portion respectively. Assays of these 4 preparations indicated that the central and the anterior portions contained more melanophore-dispersing activity than the lateral and posterior portions. In the third set of glands, therefore, 3 divisions were made consisting of (1) the extreme lateral portions, (2) the medial postero-dorsal (juxta-neural portion) and (3) the medial antero-ventral portion.

All extracts were assayed for melanophore-dispersing activity by the method of Teague[7] in which the hypophysectomized frog is used as a test-object. The results are summarized in Tables I and II. In Table I, the concentration of the hormone in various regions of the glands is expressed as units per gamma of acetone-desiccated powder (1 unit is the melanophore-dispersing activity of 1 γ of alkali-treated U.S.P. XI posterior pituitary powder.[7]). In Table II, the hormone concentrations are expressed in percentage values, the antero-ventral portion being given the arbitrary value of 100.

The most striking finding is the comparatively low concentration of melanophore-dispersing hormone in the postero-dorsal (juxta-neural) region of both the armadillo and cetacean glands. This area corresponds topographically to the pars intermedia of other species. The highest concentration of the hormone was found in the antero-ventral region. The differences between these 2 regions are most marked in the dolphin and the armadillo glands, which were fixed

---

[7] Teague, R. S., *Endocrinology*, 1939, **25**, 962.

TABLE I.

	Dolphin.				
	A	B	Finback	White Whale	Armadillo
Antero-ventral region	1.0	3.3	0.028	0.111	5.0
Postero-dorsal "	0.08	0.4	0.017	0.045	0.86
Lateral "	0.5	2.0	0.017	0.083	0.59
Antero-dorsal "	0.4				

The hormone concentration is expressed as units per γ of acetone desiccated powder.

TABLE II.

	Dolphin.				
	A	B	Finback	White Whale	Armadillo
Antero-ventral region	100	100	100	100	100
Postero-dorsal "	9	12	61	41	17
Lateral "	50	61	61	75	12
Antero-dorsal "	50				

The hormone concentration of the antero-ventral region is given the value of 100, and the concentration in other regions is expressed as % of this value.

in acetone immediately after the death of the animals, and assayed soon after fixation. In the case of the white and finback whales, the glands could not be removed for several hours after death, hence postmortem diffusion may have occurred. The lower absolute values for hormone content in these species may be due in part to autolysis, or in the case of the finback whale to deterioration, since this gland had been stored in acetone for 4 years.

Discussion. Our results are in accord with those of Kleinholz and Rahn[8] and DeLawder, Tarr and Geiling[9] on the distribution of the melanophore-dispersing hormone in the chicken hypophysis.

Histological examination of the hypophysis of the armadillo and of the white whale reveals no obvious cytological differences between the anterior and posterior regions of the anterior lobe. Wislocki[1] and Wislocki and Geiling[2] report that in the bottlenose porpoise (dolphin) and the finback, sperm and blue whales, the anterior lobe is relatively homogeneous in character and no residual cleft is present. Recently, however, we have examined a freshly-fixed gland of the bottlenose porpoise. In this specimen, there is a large colloid-filled cleft in the ventral region of the anterior lobe. In order to determine whether this represents the residual lumen of Rathkes pouch, the embryonic development must be studied. We have a number of embryonic glands at our disposal and from the study of these we hope to throw further light on this problem.

---

[8] Kleinholz, L. H., and Rahn, H., *Proc. Nat. Acad. of Sci.*, 1939, **25**, 145.

[9] DeLawder, A. M., Tarr, L., and Geiling, E. M. K., *J. Pharm. and Exp. Therap.*, 1934, **51**, 142.

Summary. In the cetacean and armadillo hypophysis, the melanophore-dispersing hormone is found in greatest concentration in the antero-ventral region. The postero-dorsal (juxta-neural) region contains the lowest concentration. These findings accord well with those of DeLawder, Tarr and Geiling and of Kleinholz and Rahn in the chicken hypophysis, in which the highest concentration of melanophore-dispersing activity was found in the anterior region of the gland.

11214 P

Whealing Response of Human Skin to Ultraviolet Light and the Histamine Theory of Allergic Reactions.*

HAROLD A. ABRAMSON.

From the Biological Laboratory, Cold Spring Harbor, Long Island, and the Mount Sinai Hospital, New York City.

Although a whealing response to ultraviolet light has been reported previously, there is apparently no record of the spectral region of hypersensitiveness in the individuals who comprise the group hypersensitive to ultraviolet light. For the past 3 years a case of this type *(Urticaria solare)* has been studied and the following data may now be reported.

(1) The region of spectral hypersensitiveness (in this woman of 32 years) as manifested by whealing begins very close to 3700 Å and extends to lower wave lengths with increasing sensitiveness in the region where absorption occurs. This zone corresponds to the zone of response of the skin to pigmentation reported by Luckiesh and Taylor.[1] The syndrome, therefore, might be connected with the pigmentation process, although pigmentation may be induced in the usual way. The case studied by Blum, *et al.*,[2] is probably another entity for his case responded in the visible region. The statement by Laurens that *Urticaria solare* is produced by luminous violet and blue rays from 3900 to 5300 Å must evidently be modified.[3]

---

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Luckiesh, M., and Taylor, A. H., *J. A. M. A.*, 1939, **112**, 2510.

[2] Blum, H. F., Allington, H., and West, R., *J. Clin. Invest.*, 1935, **14**, 435.

[3] Laurens, H., *J. A. M. A.*, 1938, **111**, 2385.

(2) Observations of the development of over 500 wheals to various sources of light have shown that pseudopods never form. The borders of the wheal always initially coincide exactly with the area irradiated and the wheals last several hours if the stimulus is sufficient. Spread of the wheal is slight but uniform and is probably due to an increase in intradermal pressure by an excess of extravascular liquid. Even when wheal formation is retarded because of a low dosage of radiation, the pattern of the wheal matches that of the zone irradiated. The non-development of pseudopods is not in accord with the theory that a readily diffusible H-substance like histamine is liberated in the tissues subsequent to irradiation.

(3) An irradiated zone of the skin which had many needle punctures was treated with wet cotton and the negative pole to electrically transfer histamine from the skin during the whealing process by reversed electrophoresis of histamine. A whealing substance was not isolated from the skin by this technic although histamine itself may under certain conditions be recovered in this way.[4]

(4) A large area of the back was irradiated, producing a wheal

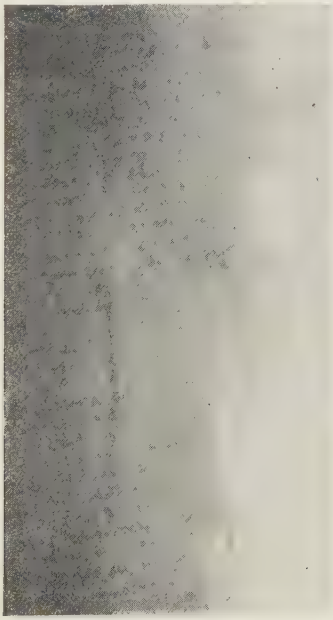


FIG. 1.

The vertical part of the cross is a sharp-edged wheal produced by ultraviolet light. The horizontal part is a superimposed histamine wheal produced by electrophoresis of histamine.

---

[4] Abramson, H. A., and Gorin, M. H., *J. Physical Chem.*, 1939, **43**, 335.

of approximately 200 in^2. without any symptoms of histamine poisoning. Evidently, a diffusible substance like histamine was not liberated in any quantity sufficient to account for the massive wheal.

(5) A histamine wheal may be readily formed (electrophoresis or injection) over an irradiated area of the skin which has responded by whealing. (Fig. 1.) In other words, the whealing response to light does not prevent histamine whealing in the same area. Similarly, a light wheal may be superimposed on a wheal formed by electrophoresis of 1:10,000 histamine.

Summary. (1) The spectral response to light in a case of *Urticaria solare* may be connected with the pigmentation mechanism. (2) A process which produces a relatively non-diffusible whealing substance is probably responsible for the whealing in the case in question.

The preceding observations, which will be reported in detail elsewhere, do not support the point of view that histamine or a readily diffusible H-substance of low molecular weight is responsible for the skin response to ultraviolet irradiation.

11215

Toxin Production by *C. diphtheriae* Types: Mitis, Gravis and Intermedius.

FRANK CHARLES HAPPOLD.*† (Introduced by J. H. Mueller.)

From the Department of Bacteriology and Immunology, Harvard University Medical School.

The differentiation of *C. diphtheriae* into 3 groups, gravis, intermedius and mitis, and the observation that the case death rate was highest and the incidence of paralysis greatest with gravis type infections, less with intermedius and least with mitis infections,[1, 2] coupled with reports of the inability of gravis strains to produce potent toxins,[3] have led to the postulation of two theories to explain the apparent anomaly: (a) That gravis strains have a more rapid invasive power than other strains.[4] (b) That the gravis type is more toxigenic *in vivo* than *in vitro* compared with the intermedius and mitis types.

---

* Leverhulme Research Fellow.

† I wish to thank the staff of the Department of Bacteriology and Immunology of Harvard University Medical School for their hospitality during the course of these studies, and in particular, Dr. J. Howard Mueller for his interest and encouragement. My thanks are also due to the Leverhulme Fellowship trustees who supported this work.

[1] Anderson, Happold, McLeod and Thomson, *J. Path. and Bact.*, 1931, **34**, 667.

[2] Cooper, Happold, McLeod and Woodcock, *Proc. Roy. Soc. Med.*, 1935, **29**, 5.

The studies of Mueller[5] and Mueller and Pappenheimer[6] resulted in the production of chemically-defined media for the production of diphtheria toxin, and their findings have been applied in comparative studies of toxin formation. It is conceivable that modification of media may affect the toxin production by different types to different degrees and if this is so, it would give support to the second hypothesis. In addition the knowledge which is accumulating in the studies which are in progress at Harvard and at Leeds, that the nutritional requirements of gravis and intermedius types are not as simple as are those of the mitis, increases the possibility that additional toxic elements not previously recognized may be produced by gravis and intermedius strains.

Experimental. Media. The basal medium used was prepared as described by Mueller.[5] All glassware was cleaned with chromic acid before use. Media was sterilized by autoclaving at 10 lb pressure for 10 min.

Strains. Ten gravis, 8 intermedius, 4 mitis and 3 "Park Williams 8" strains were used. Of these 7 gravis and 4 intermedius strains were known to be from fatal or severe infections.

Flasks were inoculated from 24-hour peptone cultures since the inoculation from plate cultures results in poor and irregular toxin formation. Duplicate cultures were tested for toxin after 3, 5, 7 and 9 days' growth. The growth of mitis strains was good, of gravis, moderate, of intermedius infinitesimal. The latter produced less than 2½ Fl units of toxin.

Gravis—Six strains (severe) produced 5-10 Fl units per ml.
Four strains produced <2½ units per ml.
Mitis—One strain (severe) produced 10 Fl units per ml.
Three strains produced <2½ units per ml.
Park 8 (Toronto) produced 60 Fl units per ml and the δ*L3* and δ*1058* strains 17 units.

Toxin production in complex media. Strains have been tested for toxin production in a further variety of media, after 3, 5, 6 and 8 days. (a) Parke Davis Peptone water (corrected for Fe content), (b) Wheeler and Wadsworth's Medium, and (c) Media (9) and (7) modified by the addition of sterile rabbit's serum (1 ml per 30 ml) both before and after heat sterilization. Only the 3 Park 8 strains produced toxin on these media and their titer of toxin was inferior to that produced on medium (7).

---

[3] Parish, Watley and O'Brien, *Brit. Med. J.*, 1932, ii, 915.

[4] Orr, McLeod and Woodcock, *J. Path. and Bact.*, 1939, **48**, 99.

[5] Mueller, *J. Bact.*, 1938, **36**, 499.

[6] Pappenheimer, Mueller and Cohen, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 795.

Neither free growth nor considerable pellicle formation in cultures of pathogenic strains of *C. diphtheriae* necessarily leads to the production of toxin even when there is an absence of wide pH fluctuation during growth.

Iron Requirements. The iron content of the basal medium was varied to contain 6 γ, 9 γ, 12 γ, and 24 γ $FeSO_4 \cdot 7H_2O$ per 30 cc. (The Park 8 strains had left a considerable proportion of their inorganic iron in the media after reaching the point of optimum toxin production, whereas gravis and mitis strains rapidly cleared the medium of inorganic iron.) PWδ1058 produced most toxin with 6 γ $FeSO_4 \cdot 7H_2O$ per 30 cc, δL3 and Toronto with 12 γ.

Addition of Mineral Constituents. In view of the findings of Evans, Happold and Handley that additional mineral requirements were necessary for the growth of intermedius strains, a salt mixture was prepared, containing those inorganic constituents which appeared in small concentrations in the original medium devised in this investigation for these strains.

The growth was increased by the addition of concentrations of this mixture equivalent to their content in (7), but only 2, Shore and T626, gave toxin in this new media (5-10 Fl per ml) and that with irregularity.

Energy Sources. The following energy sources were substituted in variable concentrations for the 2% Maltose used in medium (9), *viz.*, glucose, sodium citrate, glycerol, sodium lactate and sodium pyruvate. Toxin formation was inhibited by the lactate, pyruvate and citrate, perhaps because the cultures developed a strong alkalinity. Toxin formation was lessened by the substitution of the glucose and glycerol. These cultures developed an increased acidity in comparison to the maltose cultures.

The effect of balancing the increased acidity due to the fermentation of the glucose and glycerol with the alkalinity from the sodium lactate was now tried. Better growth of cultures was obtained for intermedius strains but the "non-toxin"-producing strains were not induced thereby to produce toxin.

Effect of Certain Amino Acids. Tryptophan. In the preparation of medium (9) all but traces of tryptophan must be destroyed. The effect of added tryptophan (1-5 mg per 10 ml of media) was tested on the 3 Park 8 strains. The toxin production of the Toronto strain was unaffected by such addition, δ13 and δ1058 produced more toxin subsequent to such addition, the effect being greatest with δ1058.

Isoleucine was also shown to be an essential amino acid for the growth of the intermedius type.[7] Its effect on toxin production was studied. The intermedius strain "Shore" produced more toxin after such enrichment. The Park 8 strains were unaffected by such addition.

Basal medium	5 Fl
” ” + 3 mg isoleucine per 30 cc	5-7 Fl
” ” + 30 ” ” ” ” ”	10-15 Fl

Pantothenic Acid. It was shown[8] that pantothenic acid was an essential growth factor for gravis strains (unessential for all mitis strains tested). It is also an essential for certain intermedius strains. Its optimum effect lies between 1 and 5 γ pantothenic acid per 10 cc medium. The addition of pantothenic acid to cultures of gravis and intermedius strains inoculated from 18-hour peptone cultures into medium[9] or the same enriched with tryptophan or isoleucine, increases the rate of growth but does not cause greater toxin production.

Conclusions. The Park 8 strains are the only potent toxin producers from the gravis, intermedius, mitis and Park 8 strains tested. All strains were virulent, the Park 8 and Freeman strains but feebly so. The cultural requirements of the Park 8 strains for optimum toxin production vary with the strain in its iron and tryptophan requirements. The same is true for the intermedius strain "Shore", whose toxigenic capacity is also increased by the addition of isoleucine.

Whilst all markedly pathogenic strains of *C. diphtheriae* tested were poor toxin producers under the experimental methods applied, there is indication that optimum conditions may not have existed for toxin production by these strains.

---

[7] Evans, Happold and Handley, *Brit. J. Exp. Path.*, 1939, **20**, 41.

[8] Evans, Handley and Happold, *Brit. J. Exp. Path.*, 1939, **20**, 396.

[9] Mueller, *J. Immunol.*, 1939, **37**, 103.

11216

Inactivation of Intracellular Phage Precursor by Iodoacetic Acid.*

A. P. KRUEGER AND E. J. SCRIBNER.

From the Department of Bacteriology, University of California, Berkeley, California.

There exists experimental evidence for the view that phage production involves the reaction: Inactive intracellular phage precursor + phage ⟶ phage.[1] The phage precursor has been shown to develop whenever the environmental conditions favor active bacterial metabolism and growth.[2] We term cells which contain a store of precursor "activated bacteria", and have found that the precursor content of the bacteria is very thermolabile, being destroyed at rapid rates when the cells are heated to 45°-50°C.[3] At these temperatures destruction of the precursor takes place long before sufficient damage is done to the cells to destroy their reproductive mechanism. The critical thermal increment for heat inactivation is 90,000, suggesting that the precursor either is a protein or contains a protein.

In the present paper we wish to report experiments in which destruction of precursor was brought about by concentrations of iodoacetic acid that were not lethal for the bacteria used.

Activated staphylococci were prepared by growing the organisms in a heavily oxygenated medium.[2] The activated cells were separated from the broth, resuspended in phosphate buffer solution of pH 6.1 and were stored for 1 hour at 5°C before using. Four ml aliquots of activated cell suspension were added to 1 ml amounts of varying concentrations of iodoacetic acid. The mixtures were kept at 5°C for given periods of time after which aliquots were tested for the presence of phage precursor and for the numbers of viable bacteria per ml. The precursor test was done by adding 4 ml amounts of cell suspension to 1 ml of phage solution containing 1×10^9 activity units/ml.[4] The test suspensions were kept at 5°C for 6 minutes to allow interaction of phage and residual activated cells after which they were diluted for titration. Controls consisted of: (a) Activated bacteria

---

* Acknowledgment is made to the John and Mary R. Markle Foundation for their generous support through Grant-in-aid of Research for this program.

[1] Krueger, A. P., and Mundell, J., *Science*, 1938, **88**, 550.

[2] Krueger, A. P., and Scribner E. J., *J. Gen. Physiol.*, 1939, **22**, 699.

[3] Krueger, A. P., Mecraken, T., and Scribner, E. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 573.

[4] Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 557.

maintained at 5°C and pH 6.1 in phosphate buffer without iodoacetic acid for 30- and 60-minute periods. When subsequently tested by the addition of phage it was found that the precursor content of the cells had not diminished. (b) Known amounts of phage were exposed to concentrations of iodoacetic acid varying from M/1,000 to M/100,000 in phosphate buffer pH 6.1 at 5°C for varying periods. These concentrations of iodoacetic acid did not diminish phage titers during periods of exposure up to 1 hour.

The results of these experiments are summarized in Table I and show that appreciable inactivation of phage precursor is produced by as little as M/10,000 iodoacetic acid acting on activated bacteria for 15 minutes. During this time no measurable number of cell deaths occurred as determined by the plate count.

Since phage precursor is readily destroyed by concentrations of iodoacetic acid insufficient to kill staphylococci, it was thought that in a mixture of bacteria and phage a dilution might be found which would serve to completely inhibit phage production while permitting bacterial growth. In 15 experiments the rates of phage production and bacterial growth were followed in the presence of concentrations of iodoacetic acid ranging from M/4,000 to M/20,000 with $[\text{Bacteria}]_0 = 2 \times 10^7$ $[\text{Phage}]_0 = 1 \times 10^8$, at pH 7.2 and 6.1 and temperature 36°C. The rates of phage production were materially lowered in the iodoacetic acid mixtures and it took longer for lysis to occur. The end titers likewise were lower, averaging 25% of the

TABLE I.

Inactivation of intracellular phage precursor by CH_2I COOH. Activated Staphylococci were exposed to different concentrations of CH_2I COOH for varying periods of time. Samples were tested for phage precursor and for the number of viable bacteria per ml.

Molarity CH_2I COOH	Exposure (minutes)	Bacteria $\times 10^9$/ml	$[\text{Phage}]_0$	[Phage] Final
Control	0	4.6	2×10^8	1.4×10^9
.0001	1.0		"	1.0 "
.0001	3.0		"	1.0 "
.0001	5.0		"	1.0 "
.0001	15.0	2.5	"	4.0×10^8
.0001	30.0	3.8	"	3.7 "
.0001	60.0	4.1	"	2.8 "
Control	0	1.3	"	1.0×10^9
.00025	5.0		"	1.0 "
.00025	15.0	1.0	"	3.3×10^8
.00025	30.0	3.2	"	2.6 "
Control	0	6.0	"	1.1×10^9
.0005	5.0		"	4.5×10^8
.0005	15.0	8.2	"	3.0 "
.0005	30.0	4.7	"	2.2 "

control titers. Unfortunately the rate of bacterial growth likewise was reduced by the iodoacetic acid and it is therefore not possible to interpret the results as being due to the inactivation of precursor by iodoacetic acid. They could equally well arise from the effect of iodoacetic acid on bacterial growth with resultant lowering of the number of precursor-producing units available.

To summarize, it has been shown that concentrations of iodoacetic acid ranging from M/2,000 to M/10,000 serve to abolish the phage-augmenting capacity of activated staphylococci (organisms containing phage precursor) at 5°C and pH 6.1, without killing them.

11217 P

Quantitative Determination of Pneumococcal Capsular Polysaccharide by Photronreflectometric Titration of Precipitation with Excess Antibody.*

SAMUEL CHARLES BUKANTZ† AND JESSE G. M. BULLOWA.

From the Littauer Pneumonia Research Fund, New York University College of Medicine, and the Medical Service, Harlem Hospital, Department of Hospitals, New York City.

Several recent publications[1, 2] have again dealt with the occurrence of capsular polysaccharide (SSS) in the blood of pneumococcal pneumonia patients and the serious prognosis usually associated with this finding. A number of observers in the past[3-10] have called attention to the relationship of SSS to the virulence of pneumococci and to its ability to "neutralize" immune bodies specifically. Despite the well-developed knowledge of the effect of SSS and its clinical significance, little is known of the conditions governing its production by pneumococci either *in vivo* or *in vitro*. A simple, accurate method for the quantitative determination of the substance is essential to the pursuit of such studies and additional studies on the effect of sulfonamide compounds on the elaboration of SSS. Heidelberger and Kendall[11] utilized a standardized solution of antibody which had been titrated with varying dilutions of known SSS to obtain a curve of precipitates determined by micro-Kjeldahl procedures, precautions being taken to work with purified SSS and to remain within the zone of antibody excess. This procedure is not easily adapted to the studies planned. Hooker and Boyd[12] observed that, in regions of considerable antibody excess, times of flocculation varied inversely and in the same ratio as changes in concentration of antigen. By applying this formulation they were able to estimate small quantities of precipitinogen present in solutions of unknown concentration. Recently, Libby[13] developed the photronreflectometer which he described as useful for the detection of unknown solutions of SSS by titrating a standard serum with varying dilutions of the unknown until predetermined equivalents of unit and galvanometer reading are obtained.

---

* These studies received financial support from the Metropolitan Life Insurance Company, and from Mr. Bernard H. Baruch, Mr. Bernard M. Baruch, Jr., Miss Belle N. Baruch, and Mrs. H. Robert Samstag.

Technical assistance in this work was rendered by Miss Anita Cooper.

† Littauer Fellow in Pneumonia Research.

[1] Bukantz, S. C., Bullowa, J. G. M., and de Gara, P. F., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 250.

[2] Bullowa, J. G. M., Bukantz, S. C., and de Gara, P. F., *Ann. Int. Med.*, in press.

[3] Rosenow, E. C., *J. Inf. Dis.*, 1907, **4**, 285.

[4] Cole, R., *J. Exp. Med.*, 1917, **26**, 453.

[5] Felton, L. D., and Bailey, G. H., *J. Inf. Dis.*, 1926, **38**, 131.

[6] Woo, S. T., *J. Exp. Med.*, 1926, **43**, 623.

[7] Sia, R. H. P., *J. Exp. Med.*, 1926, **43**, 633.

[8] Sickles, G. M., *J. Inf. Dis.*, 1927, **40**, 369.

[9] Ward, H. K., *J. Exp. Med.*, 1932, **55**, 511; 1932, **55**, 519.

[10] Spring, W. C., Lowell, F. C., and Finland, M., *J. Clin. Inv.*, 1940, **19**, 163.

In the present investigation a commercially prepared type III pneumococcal rabbit antiserum (Lederle), having approximately 4,000 units per ml, or 3.75 mg of precipitable nitrogen per ml was diluted with ether-phenol saline solution to a concentration of 25 units per ml or .023 mg precipitable N per ml. By testing supernates of a series of reactions between capsular polysaccharide and this antibody solution, the equivalence point concentration of capsular polysaccharide was found to be .004 mg. A dried preparation of purified polysaccharide III‡ was accurately weighed§ and volumetrically diluted to 1 to 100,000 (.01 mg per ml). Volumetrically accurate dilutions ranging between .0005 and .005 mg per ml, and differing by .0005 mg concentrations, were then made, using a saline diluent. Using volumetric pipettes, 1 ml of each of these dilutions

---

[11] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1932, **55**, 555.

[12] Hooker, S. B., and Boyd, W. C., *J. Gen. Physiol.*, 1935, **19**, 373.

[13] Libby, R. L., *J. Immunol.*, 1938, **34**, 71, 268.

‡ Kindly provided by Dr. W. G. Malcolm and by Dr. Rachel Brown.

§ For the weighing of the samples we are indebted to Dr. Joseph B. Niederl and to Victor Niederl of the microanalytical laboratories at Washington Square College of New York University.

TABLE I.

Turbidity readings of reaction between antipneumococcal rabbit serum III 473h273h (1-160 dilution, .023 mg precipitable N per ml) and SSS III (R. Brown t-16) in saline solution. Incubated 20 minutes at 37°C. Sensitivity of photronreflectometer at 15.

Photronreflectometer readings	Mg capsular polysaccharide [per ml saline]																			
	.005		.0045		.004		.0035		.0030		.0025		.0020		.0015		.001		.0005	
Dry blank reading	2.5	3.0	2.0	2.5	2.5	2.0	3.0	2.0	2.0	3.0	3.0	2.5	2.5	3.0	2.5	2.0	2.5	2.5	3.0	2.5
Reading after 20 min	30.0	31.0	29.5	31.0	31.5	30.5	31.5	29.5	29.0	30.0	28.5	28.0	24.0	24.5	15.0	15.5	12.5	12.5	8.0	7.5
Corrected reading	27.5	28.0	27.5	28.5	29.0	28.5	28.5	27.5	27.0	27.0	25.5	25.5	21.5	21.5	12.5	13.5	10.0	10.0	5.0	5.0
Avg reading (nearest .5)	28.0		28.0		29.0		28.0		27.0		25.5		21.5		13.0		10.0		5.0	

was mixed with 1 ml of the standard serum[‖] in refraction cells, incubated in a water bath (totally immersed) at 37°C for 20 minutes,[¶] and the resulting turbidities read, in duplicate, in the photronreflectometer. Table I gives the readings obtained in such a determination, performed in duplicate. Readings checked, on repetition, with a high degree of accuracy, variations of not greater than 0.5 to 1.0 galvanometric deflection being observed for each point determined. Fig. 1 is a curve prepared from 5 such determinations. The concentration of an unknown solution can be determined by reference of experimental titrations to this curve. In titrating an unknown solution, precaution is always taken to assure the use of a dilution which will be in the range of antibody excess with the standardized serum, *i. e.,* in the zone to the left of "equivalence point concentrations" in Fig. 1 (this can also be checked by testing the supernate of the tube after the reaction is brought to completion by refrigeration and centrifugation). The method seems well-adapted to compara-

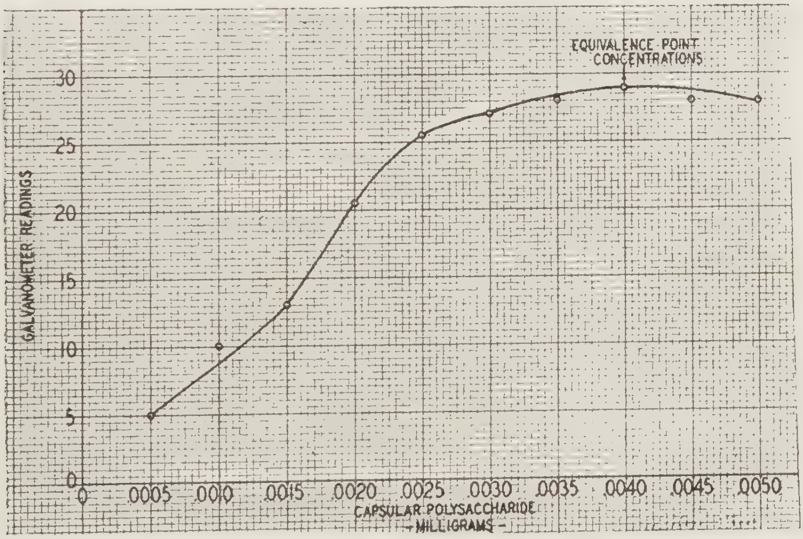


Fig. 1.

Smoothed graph of photronreflectometric readings (average of 5 determinations) obtained by titrations of varying quantities of capsular polysaccharide III (Dr. Brown T-16) with rabbit antipneumococcal serum III (containing .023 mg precipitable N per ml).

[‖] The serum was not absorbed with "C" substance or somatic nucleoprotein; however, filtrates of cultures of heterologous pneumococci (4 and 21) failed to react with the serum.

[¶] Preliminary experiments (unpublished) by Dr. Ely Perlman, of this laboratory, indicated this to be a satisfactory concentration.

tive determinations for a single preparation of SSS. Since it may be expected that a given strain of pneumococcus produces a uniform SSS, *in vitro,* comparative determinations of SSS produced by such a single strain under differing conditions of growth, using the same serum, would appear to be valid. In such instances, the standard curve should be prepared with dilutions of SSS made in broth.

Summary. A standard solution of antipneumococcal rabbit antibody type III was titrated with varying dilutions of capsular polysaccharide, in the zone of antibody excess. Photronreflectometric readings were made after 20 minutes of incubation at 37°C and a standard curve made. Unknown concentrations of SSS can be determined by identical titrations of varying dilutions with reference to the standard curve, provided a single system is utilized.

11218

A Micromortar Especially Adapted to Virus Studies in Insects.*

JOHN C. BUGHER. (Introduced by J. H. Bauer.)
From the Yellow Fever Laboratory at Villavicencio, Colombia.

During efforts to improve quantitative methods of studying yellow fever virus in arthropods, it became evident that a single insect, such as a mosquito, can not be triturated in the ordinary mortar without the loss of much of the material, particularly when only a little liquid is added. The use of a small mortar that would also serve as a centrifuge-tube would reduce the loss and simplify the technic.

A device designed to achieve these ends was made of Pyrex glass; the two parts were first formed as accurately as possible in a flame; then the pestle was ground on a wheel to an approximate fit with the apposing surface of the mortar. The grinding surface was then formed at the bottom of the tube by seating the pestle B into A, using carborundum mixed with turpentine or glycerol. The grinding should be finished with a fine powder to give a reasonably fine tooth to the finished surface.

It is essential that the two grinding surfaces be well fitted and that the clearance established by the neck of the pestle should exceed in

---

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the Section of Special Studies maintained by the Colombian Government and the International Health Division of the Rockefeller Foundation.

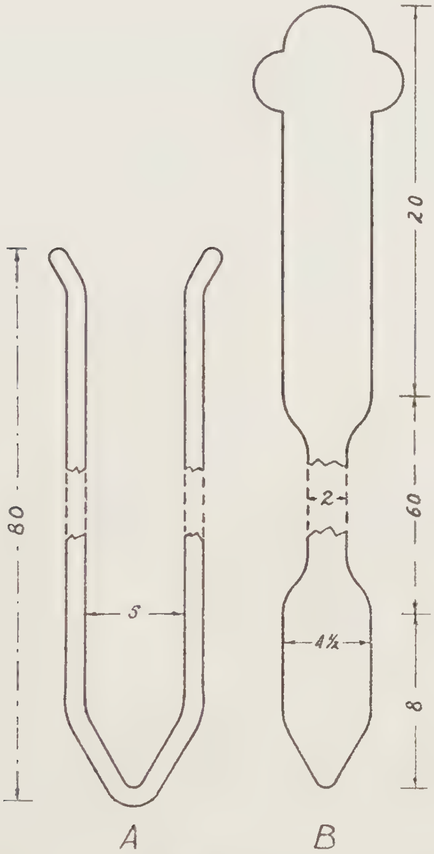


FIG. 1.

Construction-details of micromortar A and pestle B. Dimensions given are in millimeters. Angle of grinding cone should be approximately 60°.

volume the quantity of fluid to be used in the mortar when the pestle is in place.

In the final grinding with carborundum, almost inevitably a ground surface will be formed in the upper portion of the tube where the head of the pestle is inserted. This is an advantage in that the presence of this ground surface assists in matching a particular pestle with its mortar. For further convenience, the exterior of the mortar may be etched to mark the volumes desired.

Mode of Use. The insect is dropped to the bottom of the dry mortar. The end of the pestle is moistened with the fluid in which the ground insect is to be suspended and is inserted so that the insect is caught between the two matching surfaces of the mortar and pestle. Grinding by revolving the pestle is continued until no more particles are visible. Frequently the addition of more diluent is desirable. When grinding is finished, the pestle is lifted and the additional fluid runs down the stem, thus washing adhering material from the pestle and from the wall of the mortar.

After centrifugation of this tubular mortar the supernate is allowed to run toward the mouth of the tube, where it can be collected in a graduated syringe or pipet.

These pestles and mortars are obviously not interchangeable, so it is desirable that matched parts be suitably numbered. The assembled micromortars may be tubed, and sterilized by dry heat.

11219 P

Inhibitory Effect of Implanted Estrogenic Hormone Crystals upon Post-Menopause and Castration Hypophysis of Women.

UDALL J. SALMON, SAMUEL H. GEIST AND ROBERT I. WALTER.*
(With the technical assistance of A. A. Salmon.)
From the Laboratories of the Mt. Sinai Hospital, New York City.

It has previously been shown in animals[1-8] that after castration there is an increased secretion of gonadotropic hormone by the pituitary. Similar gonadotropic hyperactivity of the pituitary was shown to occur in humans after castration and after the menopause.[9-14] It

---

[*] Joseph Brettauer Research Fellow in Gynecology.

[1] Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101.

[2] Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **89**, 371.

[3] Martins, T., *Insti. Oswaldo Cruz. Supplem. das Memor.*, 1929, **10**, 242.

[4] Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 702.

[5] Moore, C. R., and Price, D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 38.

[6] Kallas, H., *Klin. Wchnschr.*, 1930, **9**, 1345.

[7] Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., *Endocrinology*, 1932, **16**, 655.

[8] Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

[9] Fluhmann, C. F., *Am. J. Obs. and Gyn.*, 1929, **20**, 1.

[10] Zondek, B., *Klin.-therap. Wchnschr.*, 1930, **9**, 393.

[11] Osterreicher, W., *Klin. Wchnschr.*, 1933, **12**, 538.

[12] Hamburger, C., *Acta Path. et Microbiol. Scand.*, 1933, **17**, 1.

[13] Frank, R. T., Salmon, U. J., and Friedman, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1666.

[14] Salmon, U. J., and Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1236.

has furthermore been shown that this hyperactivity can be inhibited by the administration of adequate amounts of estrogens.[15-18] In humans, the inhibition was demonstrated by a decrease or disappearance of the gonadotropic hormone in the urine after estrogen administration. In a previous report[19] it has been shown that by administering estrogens in the form of α-estradiol benzoate crystals implanted under the skin, a therapeutic effect is obtained which is strikingly more prolonged than that which results from a comparable amount of the hormone given intramuscularly in oil. This prolongation of the therapeutic effect was attributed to a retardation of the rate of absorption of the hormone.

The study reported here was undertaken to determine the effect of the implanted crystals of estrogenic hormone on the gonadotropic hormone excretion in surgical castrates and post-menopausal women and to compare the duration of the inhibitory effect upon the hypophysis with that resulting from estrogens administered in oil.

Eight women were selected (5 surgical castrates and 3 spontaneous menopause), all of whom were found to excrete castrate gonadotropic hormone (c.g.h.), the amounts varying from 5 to 28 R.U. per day. Seven of these were implanted subcutaneously with crystalline α-estradiol† or α-estradiol benzoate,† in doses varying from 25 to 50 mg. One patient (a surgical castrate) was given 10 mg daily of α-estradiol benzoate in solution in oil,† intramuscularly, for 6 days. Gonadotropic hormone urine studies were performed on 24- and 48-hour specimens throughout the entire experimental period, which varied from 26 to 92 days.

For the determination of c.g.h. in the urine, a modification of the acetone precipitation method was employed. 600 cc of urine of each specimen were extracted Extracts representing 200 and 100 cc were injected into immature female rats weighing 30 to 35 g, in divided doses, over 3 days. Animals were sacrificed at the end of 96 hours and serial sections made of the ovaries. The smallest amount of extract which induced follicle stimulation (minimum of 3 large

---

[15] Frank, R. T., and Salmon, U. J., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 311.

[16] Salmon, U. J., and Frank, R. T., Proc. Soc. Exp. Biol. and Med., 1936, **33**, 612.

[17] Albright, F., *Endocrinology,* 1936, **20**, 24.

[18] Jones, M. S., and MacGregor, T. N., *Lancet,* 1936, **2**, 974.

[19] Salmon, U. J., Walter, R. I., and Geist, S. H., *Science,* 1939, **90**, 162.

† We are indebted to Dr. Erwin Schwenk, of the Schering Corporation, Bloomfield, N. J., for his kindness in supplying us with the estrogens used in this investigation.

cystic follicles) or corpora lutea, was considered to contain 1 R.U. A specimen was considered negative for c.g.h. if the equivalent of 200 cc of urine failed to produce microscopic evidence of gonadotropic effect in serial sections of the rat ovaries.

Results. α-Estradiol Crystals. Three cases were implanted with α-estradiol crystals (25, 46.5, and 50 mg, respectively). Castrate gonadotropic hormone disappeared from the urine in all 3 cases 7 days after the implantation. In one case (implanted with 25 mg) c.g.h. did not reappear until 78 days later. In the other 2 cases the urine was still negative for c.g.h. at the end of 58 days.

α-Estradiol Benzoate Crystals. Four cases were implanted with α-estradiol benzoate crystals (26, 37, 27.2, and 30 mg, respectively). Castrate gonadotropic hormone disappeared from the urine 8, 9, 12, and 4 days, respectively, after the implantation. In one case (implanted with 26 mg), the urine remained negative for c.g.h. for 59 days. The remaining 3 cases have remained negative up to the time of the present report (56, 20, and 23 days after the implantation).

The one patient who was injected with 50 mg of α-estradiol in oil began to show signs of pituitary inhibition 5 days after the first injection and the urine remained negative for c.g.h. for 10 days after the last injection. Thereafter, c.g.h. reappeared in the urine.

Summary and Conclusions. It appears from these studies that, by means of the implantation of crystals of α-estradiol and α-estradiol benzoate, it is possible to achieve a strikingly more prolonged inhibition of the hypophysis in post-menopause and ovariectomized women than can be obtained by the injection of comparable amounts of estrogenic hormone in solution in oil. These findings are in accord with the clinical observations of the prolonged therapeutic effect resulting from the implantation of the estrogen crystals.

11220 P

An Electrophoretic Study of Tetanus Antitoxin Sera.

J. van der Scheer and Ralph W. G. Wyckoff.

From the Lederle Laboratories, Inc., Pearl River, N. Y.

In a previous paper[1] we described an electrophoretic analysis of several antipneumococcal horse sera that showed antibody activity in the γ-component. Since then 2 of the horses have been used to produce tetanal antitoxin. Subsequent electrophoretic analyses detected the appearance of a new component apparently associated with the freshly developed antitoxic activity.

Early in September, 1939, horses No. 9514 and No. 6225 began to receive tetanal toxin instead of Types 1 and 2 pneumococcal antigens. Three months later they had high antitoxin-titers. Electrophoretic analyses of sera taken then, and before immunization with toxin had begun, are reproduced in Figs. 1 to 4. Only traces of antipneumo-

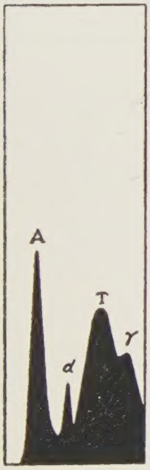


Fig. 1.

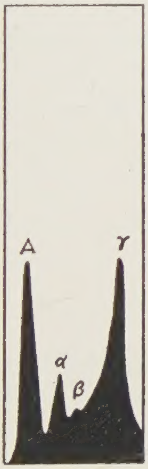


Fig. 2.

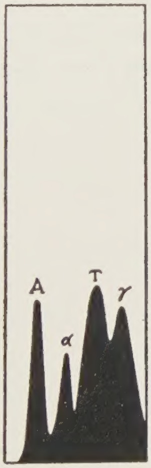


Fig. 3.

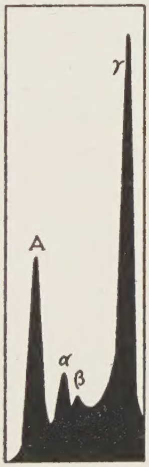


Fig. 4.

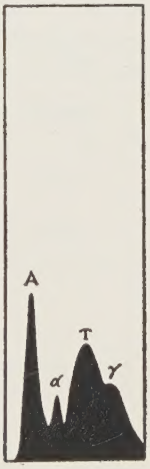


Fig. 5.

Fig. 1. Electrophoretic diagram of serum tetanal antitoxin from horse No. 9514. All sera were diluted 1:3 and dialyzed to equilibrium with a pH 7.6, 0.02M phosphate buffer that was also 0.15M with respect to NaCl.

Fig. 2. Diagram of antipneumococcal serum from No. 9514 before antitoxic immunization commenced.

Fig. 3. Diagram of serum tetanal antitoxin from horse No. 6225.

Fig. 4. Diagram of antipneumococcal serum from No. 6225 taken before antitoxic immunization began.

Fig. 5. Diagram of antitoxic serum from horse No. 7686 which had been undergoing hyperimmunization for 2½ years.

---

[1] Moore, D. H., van der Scheer, J., and Wyckoff, R. W. G., *Science*, 1939, **90**, 357.

coccal precipitin could be found in the sera taken in December and areas underlying the γ-peaks had obviously decreased greatly. With the development of antitoxin there had appeared, however, a strong new component (labeled T) with a mobility, -2.2×10^{-5} cm^2 sec^{-1} volts^{-1}, midway between β and γ. The same component was present in the strongly antitoxic serum of horse No. 756, which had never been used before for antibody-production, and in the sera of other horses producing tetanal antitoxin.

An antipneumococcal component of the same mobility was found by Tiselius and Kabat[2] in an antipneumococcal horse serum. We[1] did not observe this component in our antipneumococcal sera but inasmuch as our sera came from horses long subject to hyperimmunization we suggested that their new component might possibly be a characteristic of serum from freshly immunized horses. This, however, cannot explain the presence of the new component in the sera containing tetanal antitoxin because the serum from horse No. 7686, which has been on antitoxin production for 2½ years, also showed T as its principal component (Fig. 5). We have recently examined serum from horse No. 9514, which had been on antipneumococcal serum-production for one year; like the sera from long-immunized animals its antibody was in the γ-component (Fig. 2).

---

[2] Tiselius, A., and Kabat, E. A., *Science*, 1939, **87**, 416; *J. Exp. Med.*, 1939, **69**, 119.